UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY (EPA)
HIGH PRODUCTION VOLUME (HPV)
CHEMICAL CHALLENGE PROGRAM

ROBUST SUMMARIES DOSSIER

for

C6 MEMBERS

of the

HIGHER OLEFINS CATEGORY

Members containing C6 olefins:
CAS No. 25264-93-1, Hexene
CAS No. 68526-52-3, Alkenes C6
CAS No. 558-37-2, Neohexene

Contains Robust Summaries for the Following Substances:
CAS No. 25264-93-1, Hexene
CAS# 558-37-2, Neohexene
CAS No. 68526-52-3; Alkenes, C6
CAS No. 592-41-6, 1-Hexene
CAS No. 68526-53-4; Alkenes, C6-8, C7 rich
SHOP C68 Internal Olefin

Prepared by:

American Chemistry Council
Higher Olefins Panel

April 28, 2005

1. GENERAL INFORMATION

1.01 Details on Chemical Category

The Higher Olefins Category consists of a non-continuous range of odd- and even-numbered mono-unsaturated linear and branched olefins (C_6 through C_{54}) under 30 CAS numbers, 13 for alpha olefins and 17 for internal olefins. All CAS numbers are within the HPV Challenge Program. The $C_6 - C_{14}$ even-numbered linear alpha olefins were sponsored under the OECD SIDS program (SIAM 11). The Panel is sponsoring the C_6, C_7, C_8, C_9, C_{10}, C_{12} and C_{10-13} aliphatic linear and branched internal olefins and the C_{16} and C_{18} aliphatic linear alpha olefins in the OECD HPV Chemicals Programme (SIAM 19). The members of the category are presented below.

Members of the Higher Olefins Category

Alpha Olefins	Branched/Linear	CAS No.
Neohexene	Branched	558-37-2
1-Tridecene	Linear	2437-56-1
1-Hexadecene (ICCA)	Linear	629-73-2
1-Octadecene (ICCA)	Linear	112-88-9
1-Eicosene	Linear	3452-07-1
1-Docosene	Linear	1599-67-3
1-Tetracosene	Linear	10192-32-2
Alkenes, C10-16 alpha	Linear	68855-58-3
Alkenes, C14-18 alpha	Linear	68855-59-4
Alkenes, C14-20 alpha	Linear	68855-60-7
a-Olefin fraction C20-24 cut	Linear	93924-10-8
a-Olefin fraction C24-28 cut	Branched and Linear	93924-11-9
Alkene, C24-54 branched and linear, alpha	Branched and Linear	131459-42-2
Internal Olefins		
Hexene (ICCA)	Linear	25264-93-1
Heptene (ICCA)	Linear	25339-56-4
Octene (ICCA)	Linear	25377-83-7
Nonene (ICCA)	Linear	27215-95-8
Dodecene (ICCA – not sponsored in HPV)	Linear	25378-22-7
Alkenes, C6	Branched and Linear	68526-52-3
Alkenes, C6-8, C7-rich	No data available	68526-53-4
Alkenes, C7-9, C8-rich	Linear	68526-54-5
Alkenes, C8-10, C9-rich	Linear	68526-55-6
Alkenes, C9-11, C10-rich	Linear	68526-56-7
Alkenes, C10-12, C11-rich	Linear	68526-57-8
Alkenes, C11-13, C12-rich	Linear	68526-58-9
Heavy polymerization naphtha (petroleum)	Branched	68783-10-8
Alkenes, C10-16	Linear	68991-52-6
Alkenes, C15-C18	Linear	93762-80-2
C10,12 Olefin rich hydrocarbons	Linear	68514-32-9

C12,14 Olefin rich hydrocarbons	Linear	68514-33-0

1.1 General Substance Information

A. Type of Substance

Element [ ]; Inorganic [ ]; Natural substance [ ]; Organic [X]; Organometallic [ ]; Petroleum product [ ]

B. Physical State *(at 20°C and 1.013 hPa)*

Gaseous [ ]; Liquid [X]; Solid [ ]

C. Purity: Neohexene = 97%; Hexene is manufactured and marketed as a component of a blend, and Alkenes, C6 is a blend

1.2 Impurities

Remark: The compositions reported by manufacturers are shown below:

Hexene	25264-93-1	C6-C8 internal olefin blend: Typical composition = 1.9% C5, 43.3% C6, 21.7% C7, 31.7% C8, 1.4% C9
Alkenes, C6	68526-52-3	Typical composition: 0.5% C5 n-olefins, 1.3% C5 iso-olefins, 10.4% C6 n-olefins, 55.6% C6 iso-olefins, 3.3% C5 n-paraffins, 9.3% C5 iso-paraffins, 17.8% C6 iso-paraffins, 1.0% C7 iso-olefins
Neohexene	558-37-2	1.5% 2,3-dimethylbutene-1 (CAS No. 563-78-0), branched; related hydrocarbons

1.3 Additives

None

1.4 Synonyms

Some synonyms are: Hexene, Isomer(s)
Hexylene
Hexenes

1.5 Quantity

Remarks: Range of U.S. production volumes for 2002 submitted by Higher Olefin Panel members to Panel Manager: CAS No. 25264-93-1, Hexene = 1-10 million pounds; CAS No. 68526-52-3, Alkenes C6 = 10-50 million pounds; CAS# 558-37-2, Neohexene = 1-10 million pounds

Reference: American Chemistry Council's Higher Olefins Panel (2002)

1.6 Use Pattern

A. General Use Pattern

Type of Use:	Category:
(a) Main Industrial Use	Use in closed systems Chemical industry – chemicals used in synthesis Intermediate
Remarks:	Intermediate in the manufacture of low molecular weight fatty acids, mercaptans, plasticizer alcohols, surfactants
(b) Main Industrial Use	Non-dispersive use Chemical industry – chemicals used in synthesis Intermediate
Remarks:	Intermediate in the manufacture of low molecular weight fatty acids, mercaptans, plasticizer alcohols, surfactants
(c) Main Industrial Use	Use in closed systems Polymers industry Intermediate
Reference:	American Chemistry Council's Higher Olefins Panel (2002)

B. Uses In Consumer Products

Not applicable

1.7 Sources of Exposure

Source:

Remarks: These products are produced commercially in closed systems and are used primarily as intermediates in the production of other chemicals (including polymers). No non-intermediate applications have been identified. Any occupational exposures that do occur are most likely by the inhalation and dermal routes. It is a common practice to use personal protective equipment. In the case of dermal exposures, protective gloves would be worn due to the mildly irritating properties of this class of chemicals (ACC Higher Olefins Panel). Results from modelled data suggest that on-site waste treatment processes are expected to remove these substances from aqueous waste streams to the extent that they will not be readily detectable in effluent discharge (EPIWIN, 2000b). These substances are not on the US Toxic Release Inventory (TRI) list (NLM, 2003). These olefins will not persist in the environment because they can be rapidly degraded through biotic and abiotic processes.

Reference: American Chemistry Council's Higher Olefins Panel (2003) Personal communication.

1.8 Additional Information

A. Classification and Labelling

B. Occupational Exposure Limits

Exposure Limit Value

Type: None established
Value:

Short Term Exposure Limit Value

Value: None established

C. Options for Disposal

Remarks: Incineration, diversion to other hydrocarbon uses

D. Last Literature Search

Type of search: Internal and external
Date of search: October 2003
Remark: Medline
IUCLID
TSCATS
ChemIDplus
AQUIRE - ECOTOX

2. PHYSICAL CHEMICAL DATA

2.1 Melting Point

A. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method/
guideline followed: Calculated value using MPBPWIN version 1.40, a subroutine of the computer program EPIWIN version 3.10

GLP:	Not applicable
Year:	Not applicable

Test Conditions: Melting Point is calculated by the MPBPWIN subroutine, which is based on the average results of the methods of K. Joback, and Gold and Ogle, and chemical structure. Joback's Method is described in Joback, (1982). The Gold and Ogle Method simply uses the formula Tm = 0.5839Tb, where Tm is the melting point in Kelvin and Tb is the boiling point in Kelvin. EPIWIN program used the structure for 2-hexene.

Results

Melting point value in °C:	-120.86°C

Reliability: (2) Reliable with restrictions. The result is calculated data based on chemical structure as modeled by EPIWIN.

Flag: Key study for SIDS endpoint

References: Joback, K.G. 1982. A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. In The Properties of Gases and Liquids. Fourth Edition. 1987. R.C. Reid, J.M. Prausnitz and B.E. Poling, Eds.

EPIWIN (2000a). Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

B. Test Substance

Identity: C6-C8 Internal Olefins

Method

Method/guideline followed:	ASTM D2386
GLP:	No data
Year:	No data

Test Conditions: No data

Results

Melting point value in °C:	-50°C

Reliability: (4) Not assignable. These data were not reviewed for quality.

References: Shell Chemicals UK Ltd. Chester (cited in IUCLID)

C. **Test Substance**

Identity:	2-Hexene

Method

Method/guideline followed:	No data
GLP:	No data
Year:	No data

Test Conditions:	No data

Results

Melting point value in °C:	-141.1 to -133°C
Reliability:	(2) Reliable with restrictions. These data were obtained from a reliable secondary source.
References:	Lide, D.R. (ed.) (1998-1999) CRC Handbook of Chemistry and Physics. 79th ed. Boca Raton, FL: CRC Press Inc., p. 3-193.

D. **Test Substance**

Identity:	3-Hexene

Method

Method/guideline followed:	No data
GLP:	No data
Year:	No data

Test Conditions:	No data

Results

Melting point value in °C:	-137.8 to -115.4 °C
Reliability:	(2) Reliable with restrictions. These data were obtained from a reliable secondary source.
References:	Lide, D.R. (ed.) (1998-1999) CRC Handbook of Chemistry and Physics. 79th ed. Boca Raton, FL: CRC Press Inc., p. 3-193.

E. **Test Substance**

Identity:	CAS No. 25264-93-1, Hexene

Method

Method/

guideline followed:	No data
GLP:	No data
Year:	No data
Test Conditions:	No data
Results	
Melting point value in °C:	-98°C
Reliability:	(2) Reliable with restrictions: The result is measured data as cited in the EPIWIN database. These data were not reviewed for quality.
Flag:	Key study for SIDS endpoint
References:	EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

F. Test Substance

Identity:	CAS No. 558-37-2, Neohexene
Method	
Method/ guideline followed:	No data
GLP:	No data
Year:	No data
Test Conditions:	No data
Results	
Melting point value in °C:	-115.2°C
Reliability:	(2) Reliable with restrictions: The result is measured data as cited in the EPIWIN database. These data were not reviewed for quality.
Flag:	Key study for SIDS endpoint
References:	EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

G. Test Substance

Identity:	CAS No. 68526-52-3; Alkenes, C6
Method	

Method/ guideline followed:	Calculated value using MPBPWIN version 1.41, a subroutine of the computer program EPIWIN version 3.11
GLP:	Not applicable
Year:	Not applicable

Test Conditions: Melting Point is calculated by the MPBPWIN subroutine, which is based on the average results of the methods of K. Joback, and Gold and Ogle, and chemical structure. Joback's Method is described in Joback, (1982). The Gold and Ogle Method simply uses the formula Tm = 0.5839Tb, where Tm is the melting point in Kelvin and Tb is the boiling point in Kelvin. EPIWIN program used the structure for 1-hexene.

Results

Melting point value in °C:	-95.26°C

Reliability: (2) Reliable with restrictions. The result is calculated data based on chemical structure as modeled by EPIWIN.

Flag: Key study for SIDS endpoint

References:

Joback, K.G. 1982. A Unified Approach to Physical Property Estimation Using Multivariate Statistical Techniques. In The Properties of Gases and Liquids. Fourth Edition. 1987. R.C. Reid, J.M. Prausnitz and B.E. Poling, Eds.

EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

2.2 Boiling Point

A. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method/ guideline followed:	Calculated value using MPBPWIN version 1.40, a subroutine of EPIWIN version 3.10
GLP:	Not applicable
Year:	Not applicable

Test Conditions: Boiling Point is calculated by the MPBPWIN subroutine, which is based on the method of Stein and Brown (1994). (program used structure for 2-hexene)

Results

Boiling point value in °C:	79.21°C
Pressure:	1013
Pressure unit:	hPa

Reliability: (2) Reliable with restrictions. The result is calculated data based on chemical structure as modeled by EPIWIN.

References: Stein, S. and R. Brown (1994) Estimation of normal boiling points from group contributions (1994) J. Chem. Inf. Comput. Sci. 34: 581-587.

EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

B. Test Substance

Identity: C6-C8 Internal Olefins

Method

Method:	ASTM D68
GLP:	No data
Year:	No data

Test Conditions: No data

Results

Boiling point value:	74-120°C
Pressure:	No data
Remarks:	Upper value is for 90% distilled.

Reliability: (4) Not assignable. These data were not reviewed for quality.

References: Shell Chemicals UK Ltd. Chester, as cited in IUCLID

C. Test Substance

Identity: 2-Hexene

Method

Method/guideline followed:	No data
GLP:	No data
Year:	No data

Test Conditions: No data

Results

Boiling point value: 67.9 – 68.8°C
Pressure: No data

Reliability: (2) Reliable with restrictions. Obtained from a reliable secondary source. These data were not reviewed for quality.

References: Lide, D.R. (ed.) (1998-1999) CRC Handbook of Chemistry and Physics. 79th ed. Boca Raton, FL: CRC Press Inc., p. 3-193.

D. Test Substance

Identity: 3-Hexene

Method

Method/guideline followed: No data
GLP: No data
Year: No data

Test Conditions: No data

Results

Boiling point value: 66.4-67.1°C
Pressure: No data

Reliability: (2) Reliable with restrictions. Obtained from a reliable secondary source. These data were not reviewed for quality.

References: Lide, D.R. (ed.) (1998-1999) CRC Handbook of Chemistry and Physics. 79th ed. Boca Raton, FL: CRC Press Inc., p. 3-193.

E. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method/
guideline followed: No data
GLP: No data
Year: No data

Test Conditions: No data

Results

Boiling point value in °C:	65°C
Pressure:	1013
Pressure unit:	hPa

Reliability: (2) Reliable with restrictions. The result is measured data as cited in the EPIWIN database. These data were not reviewed for quality.

Flag: Key study for SIDS endpoint

References: EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

F. Test Substance

Identity: CAS No. 558-37-2, Neohexene

Method

Method/ guideline followed:	No data
GLP:	No data
Year:	No data

Test Conditions: No data

Results

Boiling point value in °C:	41.2°C
Pressure:	1013
Pressure unit:	hPa

Reliability: (2) Reliable with restrictions. The result is measured data as cited in the EPIWIN database. These data were not reviewed for quality.

Flag: Key study for SIDS endpoint

References: EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

G. Test Substance

Identity: CAS No. 68526-52-3; Alkenes, C6

Method

Method/ guideline followed:	Calculated value using MPBPWIN version 1.41, a subroutine of EPIWIN version 3.11
GLP:	Not applicable
Year:	Not applicable
Test Conditions:	Boiling Point is calculated by the MPBPWIN subroutine, which is based on the method of Stein and Brown (1994). EPIWIN used the structure for 1-hexene

Results

Boiling point value in °C:	69.66°C
Pressure:	1013
Pressure unit:	hPa
Reliability:	(2) Reliable with restrictions. The result is calculated data based on chemical structure as modeled by EPIWIN.
Flag:	Key study for SIDS endpoint
References:	Stein, S. and R. Brown (1994) Estimation of normal boiling points from group contributions (1994) J. Chem. Inf. Comput. Sci. 34: 581-587.
	EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

2.3 Density

A. Test Substance

Identity:	C6-C8 Internal Olefins

Method

Method:	ISO 3675
GLP:	No data
Test Conditions:	No data

Results

Type:	density
Value:	ca. 700 kg/m^3
Temperature (°C):	20°C

Reliability: (2) Reliable with restrictions. These data were not reviewed for quality but were obtained from a reliable source .

Reference: Shell Chemicals UK Ltd. MSDS, Chester

B. Test Substance

Identity: 2-hexene

Method

Method: No data
GLP: No data

Test Conditions: No data

Results

Type: density
Value: 0.6869 - 0.6772 g/cm^3
Temperature (°C): 20°C

Reliability: (2) Reliable with restrictions. These data were not reviewed for quality, but were obtained from a reliable secondary source.

Reference: Lide, D.R. (ed.) (1998-1999) CRC Handbook of Chemistry and Physics. 79th ed. Boca Raton, FL: CRC Press Inc., p. 3-193.

C. Test Substance

Identity: CAS No. 558-37-2, Neohexene

Method

Method: No data
GLP: No data

Test Conditions: No data

Results
Type: Specific gravity
Value: 0.66
Temperature (°C): 16/16°C

Reliability: (2) Reliable with restrictions. These data were not reviewed for quality but were obtained from a reliable source.

Reference: Chevron Phillips Chemical Company MSDS

2.4 Vapour Pressure

A. Test Substance

Identity:	CAS No. 25264-93-1, Hexene

Method

Method/ guideline followed:	Not reported
GLP:	Not applicable
Year:	

Test Conditions:

Results

Vapor Pressure Value:	230.6 hPa
Temperature:	25°C
Remarks:	Reported as 173 mm Hg (25°C)

Reliability: (2) Reliable with restrictions. The result is measured data as cited in the EPIWIN database. These data were not reviewed for quality.

Flag: Key study for SIDS endpoint

References: Jordan, T.E. (1954) Vapor Pressure of Organic Compounds. New York, NY, Interscience Publisher, Inc.; EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

B. Test Substance

Identity:	CAS No. 25264-93-1, Hexene

Method

Method/ guideline followed:	Calculated value using the computer program EPIWIN v. 3.10, MPBPWIN v 1.40
GLP:	Not applicable
Year:	Not applicable

Test Conditions: Vapor Pressure is calculated by the MPBPWIN subroutine, which is based on the average result of the methods of Antoine and Grain. Both methods use boiling point for the calculation. The Antoine Method is described by Lyman et al., 1990. A modified Grain Method is described

by Neely and Blau, 1985. The calculation used an experimental value for BP of 65 °C from EPIWIN database.

Results

Vapor Pressure value:	230.6 hPa
Temperature (°C):	25°C
Remarks:	Reported as 173 mm Hg

Reliability: (2) Reliable with restrictions. The result is calculated data as modeled by EPIWIN using measured data as cited in the EPIWIN database. These data were not reviewed for quality.

References:

Lyman, W.J., W.F. Reehl and D.H. Rosenblatt, Eds. (1990) Handbook of Chemical Property Estimation. Chapter 14. Washington, D.C.: American Chemical Society.

Neely and Blau (1985) Environmental Exposure from Chemicals, Volume 1, p. 31, CRC Press.

EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

C. Test Substance

Identity: CAS No. 558-37-2, Neohexene

Method

Method/ guideline followed:	Not reported
GLP:	Not applicable
Year:	

Test Conditions:

Results

Vapor Pressure Value:	574.6 hPa
Temperature:	25°C
Remarks:	Reported as 431 mm Hg (25°C)

Reliability: (2) Reliable with restrictions. The result is measured data as cited in the EPIWIN database. These data were not reviewed for quality.

Flag: Key study for SIDS endpoint

References: Yaws CL (1994) as cited in EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

D. Test Substance

Identity: CAS No. 68526-52-3; Alkenes, C6

Method

Method/ guideline followed: Calculated value using the computer program EPIWIN v. 3.11, MPBPWIN v 1.41
GLP: Not applicable
Year: Not applicable

Test Conditions: Vapor Pressure is calculated by the MPBPWIN subroutine, which is based on the average result of the methods of Antoine and Grain. Both methods use boiling point for the calculation. The Antoine Method is described by Lyman et al., 1990. A modified Grain Method is described by Neely and Blau, 1985. The calculation used an experimental value for the BP of 63.4 °C (for 1-hexene) from EPIWIN database.

Results

Vapor Pressure value: 245.0 hPa
Temperature (°C): 25°C
Remarks: Reported as 184 mm Hg

Reliability: (2) Reliable with restrictions. The result is calculated data as modeled by EPIWIN using measured data as cited in the EPIWIN database. These data were not reviewed for quality.

Flag: Key study for SIDS endpoint

References: Lyman, W.J., W.F. Reehl and D.H. Rosenblatt, Eds. (1990) Handbook of Chemical Property Estimation. Chapter 14. Washington, D.C.: American Chemical Society.

Neely and Blau (1985) Environmental Exposure from Chemicals, Volume 1, p. 31, CRC Press.

EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

2.5 Partition Coefficient (log10Kow)

A. Test Substance

Identity:	C6-C8 Internal Olefins

Method

Method:	No data
GLP:	No data
Year:	No data
Test Conditions:	No data

Results

Log Kow:	3.4 – 4.6
Reliability:	(4) Not assignable. These data were not reviewed for quality.
References:	Shell Chemicals UK Ltd. Chester, as cited in IUCLID

B. Test Substance

Identity:	CAS No. 25264-93-1, Hexene

Method

Method:	Calculated value using the computer program EPIWIN version 3.10, KOWWIN v 1.66
GLP:	Not applicable
Year:	Not applicable
Test Conditions:	Octanol / Water Partition Coefficient is calculated by the KOWWIN subroutine, which is based on an atom/fragment contribution method of Meylan and Howard (1995). Program used structure for 2-hexene.

Results

Log Kow:	3.07
Temperature (°C):	Not applicable
Reliability:	(2) Reliable with restrictions. The result was calculated based on chemical structure as modeled by EPIWIN.
Flag:	Key study for SIDS endpoint

Reference: Meylan, W. and P. Howard (1995) Atom/fragment contribution method for estimating octanol-water partition coefficients. *J. Pharm. Sci.* 84:83-92.

EPIWIN (2000a). Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

C. Test Substance

Identity: CAS No. 592-41-6, 1-Hexene

Method

Method: No data
GLP: No data
Year: No data

Test Conditions: No data

Results

Log Kow: 3.39
Temperature (°C): No data

Reliability: (2) Reliable with restrictions. Experimental result as cited in the EPIWIN database. These data were not reviewed for quality.

Flag: Key study for SIDS endpoint

Reference: Hansch C et al., 1995, as cited in EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

D. Test Substance

Identity: CAS No. 68526-52-3; Alkenes, C6

Method

Method: Calculated value using the computer program EPIWIN version 3.11, KOWWIN v 1.67
GLP: Not applicable
Year: Not applicable

Test Conditions: Octanol / Water Partition Coefficient is calculated by the KOWWIN subroutine, which is based on an atom/fragment contribution method of Meylan and Howard (1995). Program used structure for 1-hexene.

Results

Log Kow:	3.15
Temperature (°C):	Not applicable
Reliability:	(2) Reliable with restrictions. The result was calculated based on chemical structure as modeled by EPIWIN.
Flag:	Key study for SIDS endpoint
Reference:	Meylan, W. and P. Howard (1995) Atom/fragment contribution method for estimating octanol-water partition coefficients. *J. Pharm. Sci.* 84:83-92. EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

E. Test Substance

Identity:	CAS No. 558-37-2, Neohexene

Method

Method:	Calculated value using the computer program EPIWIN version 3.11, KOWWIN v 1.67
GLP:	Not applicable
Year:	Not applicable
Test Conditions:	Octanol / Water Partition Coefficient is calculated by the KOWWIN subroutine, which is based on an atom/fragment contribution method of Meylan and Howard (1995). Program used the structure for neohexene

Results

Log Kow:	3.04
Temperature (°C):	Not applicable
Reliability:	(2) Reliable with restrictions. The result was calculated based on chemical structure as modeled by EPIWIN.
Flag:	Key study for SIDS endpoint
Reference:	Meylan, W. and P. Howard (1995) Atom/fragment contribution method for estimating octanol-water partition coefficients. *J. Pharm. Sci.* 84:83-92.

EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

2.6.1 Water Solubility (including *Dissociation Constant).

A. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method/ guideline followed: Calculated value using the computer program EPIWIN v 3.10, WSKOW v 1.40

GLP: Not applicable

Year: Not applicable

Test Conditions: Water Solubility is calculated by the WSKOW subroutine, which is based on a Kow correlation method described by Meylan et al., 1996. The calculation used an estimated Log Kow of 3.07 and a melting point of -98 °C. EPIWIN used a structure for 2-hexene to estimate Log Kow.

Results

Value(mg/L) at temperature (°C): 91.78 mg/L (25°C)

Reliability: (2) Reliable with restrictions: The result was calculated by EPIWIN using estimated data.

References:

Meylan, W., P. Howard and R. Boethling (1996) Improved method for estimating water solubility from octanol/water partition coefficient. *Environ. Toxicol. Chem.* 15:100-106.

EPIWIN (2000a). Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

B. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method/ guideline followed: No data

GLP: Year:	No data
Test Conditions:	No data
Results	
Value (mg/L) at temperature (°C):	50 mg/L (20°C)
Reliability:	(2) Reliable with restrictions. Experimental result as cited in the EPIWIN database. These data were not reviewed for quality.
Flag:	Key study for SIDS endpoint
References:	Baehr, A.L. (1987) Selective transport of hydrocarbons in the unsaturated zone due to aqueous and vapor phase partitioning . Water Resources Research 23, 1926-38; EPIWIN (2000a). Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

C. Test Substance

Identity:	CAS No. 68526-52-3; Alkenes, C6
Method	
Method/ guideline followed:	Calculated value using the computer program EPIWIN v 3.11, WSKOW v 1.41
GLP: Year:	Not applicable Not applicable
Test Conditions:	Water Solubility is calculated by the WSKOW subroutine, which is based on a Kow correlation method described by Meylan et al., 1996. The calculation used a measured Log Kow of 3..39 (for 1-hexene).
Results	
Value(mg/L) at temperature (°C):	47.46 mg/L (25°C)
Reliability:	(2) Reliable with restrictions: The result was calculated by EPIWIN using measured data cited in the EPIWIN database.
Flag:	Key study for SIDS endpoint

References:	Meylan, W., P. Howard and R. Boethling (1996) Improved method for estimating water solubility from octanol/water partition coefficient. *Environ. Toxicol. Chem.* 15:100-106. EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

D. Test Substance

Identity:	CAS No. 558-37-2, Neohexene
Method	
Method/ guideline followed:	Calculated value using the computer program EPIWIN v 3.11, ECOSAR v 0.99g
GLP:	Not applicable
Year:	Not applicable
Test Conditions:	Water Solubility is calculated by the ECOSAR subroutine, which is based on a Kow correlation method described by Meylan et al., 1996. The calculation used an estimated Log Kow of 3..04(which was calculated using the structure for neohexene) and the following formula: log WaterSol (moles/L) = -0.312 - 1.02 log Kow
Results	
Value(mg/L) at temperature (°C):	32.53 mg/L (25°C)
Reliability:	(2) Reliable with restrictions: The result was calculated by EPIWIN using data estimated by the EPIWINprogram.
Flag:	Key study for SIDS endpoint
References:	Meylan, W., P. Howard and R. Boethling (1996) Improved method for estimating water solubility from octanol/water partition coefficient. *Environ. Toxicol. Chem.* 15:100-106. EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

2.6.2 Surface tension

No data available

2.7 Flash Point (Liquids)

Test Substance

Identity: C6-C8 Internal Olefins

Method

Method: ISO 2719
GLP:

Test Conditions: No data

Results

Value (°C): -26 °C
Type of test: Closed cup

Reliability: (2) Reliable with restrictions. These data were not reviewed for quality but were obtained from a reliable source .

Reference: Shell Chemicals UK Ltd. Chester, as cited in IUCLID

2.8 Auto Flammability (Solids/Gases)

No data available

2.9 Flammability

Test Substance

Identity: C6-C8 Internal Olefins

Method

Method: No data
GLP: No data

Test Conditions: No data

Result: Highly flammable

Lower flammability limit: 0.8% in air
Upper flammability limit: 6.8% in air

Reliability: (2) Reliable with restrictions. Data were not evaluated for quality but were obtained from a reliable source .

Reference: Shell Chemical Company MSDS

2.10 Explosive Properties

No data available

2.11 Oxidising Properties

No data available

2.12 Oxidation-Reduction Potential

No data available

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 Stability

A. Photodegradation

(1) Test Substance

Identity: CAS No. 25264-93-1, Hexene; CAS No. 558-37-2, Neohexene; or CAS No. 68526-52-3; Alkenes, C6

Method

Method/ guideline followed: Other: Technical discussion

Type: water
GLP: Not applicable
Year: Not applicable

Test Conditions: Not applicable

Results

Direct photolysis: In the environment, direct photolysis will not significantly contribute to the degradation of constituent chemicals in the Higher Olefins Category.

Remarks: The direct photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural

transformation (Harris, 1982a). The reaction process is initiated when light energy in a specific wavelength range elevates a molecule to an electronically excited state. However, the excited state is competitive with various deactivation processes that can result in the return of the molecule to a non excited state.

The absorption of light in the ultra violet (UV)-visible range, 110-750 nm, can result in the electronic excitation of an organic molecule. Light in this range contains energy of the same order of magnitude as covalent bond dissociation energies (Harris, 1982a). Higher wavelengths (e.g. infrared) result only in vibrational and rotational transitions, which do not tend to produce structural changes to a molecule.

The stratospheric ozone layer prevents UV light of less than 290 nm from reaching the earth's surface. Therefore, only light at wavelengths between 290 and 750 nm can result in photochemical transformations in the environment (Harris, 1982a). Although the absorption of UV light in the 290-750 nm range is necessary, it is not always sufficient for a chemical to undergo photochemical degradation. Energy may be re-emitted from an excited molecule by mechanisms other than chemical transformation, resulting in no change to the parent molecule.

A conservative approach to estimating a photochemical degradation rate is to assume that degradation will occur in proportion to the amount of light wavelengths >290 nm absorbed by the molecule (Zepp and Cline, 1977).

Olefins with one double bond, such as the chemicals in the Higher Olefins category, do not absorb appreciable light energy above 290 nm. The absorption of UV light to cause cis-trans isomerization about the double bond of an olefin occurs only if it is in conjugation with an aromatic ring (Harris, 1982a).

Products in the Higher Olefins Category do not contain component molecules that will undergo direct photolysis. Therefore, this fate process will not contribute to a measurable degradative removal of chemical components in this category from the environment.

Reliability: Not applicable

References:

Harris J C (1982a). Rate of Aqueous Photolysis. Chapter 8 in: W. J. Lyman, W. F. Reehl, and D. H. Rosenblatt, eds., Handbook of Chemical Property Estimation Methods, McGraw-Hill Book Company, New York, USA.

Zepp, R. G. and D. M. Cline (1977). Rates of Direct Photolysis in the Aqueous Environment, Environ. Sci. Technol., 11:359-366.

(2) **Test Substance**

Identity:	CAS No. 25264-93-1, Hexene

Method

Method/ guideline followed:	Calculated values using AOPWIN version 1.90, a subroutine of the computer program EIPWIN version 3.10 which uses a program described by Meylan and Howard (1993). Program used structure for 2-hexene.
Type:	air
GLP:	Not applicable
Year:	Not applicable

Results

Indirect photolysis

Sensitiser *(type):*	OH
Rate Constant:	59.0009 E-12 cm^3/molecule-sec [cis isomer]
Rate Constant:	66.6009 E-12 cm^3/molecule-sec [trans isomer]
Degradation % after:	50% after 2.175 hrs (using a 12-hr day and avg. OH conc. of 1.5 E OH/cm^3)[cis isomer]
Degradation % after:	50% after 1.927 hrs (using a 12-hr day and avg. OH conc. of 1.5 E6 OH/cm^3)[trans isomer]

Sensitiser *(type):*	Ozone
Rate Constant:	13 E-17 cm^3/molecule-sec [cis isomer]
Rate Constant:	20 E-17 cm^3/molecule-sec [trans isomer]
Degradation % after:	50% after 2.116 hrs (using avg. OH conc. of 7 E11 mol/cm^3)[cis isomer]
Degradation % after:	50% after 1.375 hrs (using avg. OH conc. of 7 E11 mol/cm^3)[trans isomer]

Reliability:	(2) Reliable with restrictions. The value was calculated data based on chemical structure as modeled by EPIWIN. This robust summary has a rating of 2 because the data are calculated and not measured.
Flag:	Critical study for SIDS endpoint
References:	Meylan, W.M. and Howard, P.H. 1993. Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. Chemosphere 26: 2293-99

EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

(3) **Test Substance**

Identity: CAS No. 558-37-2, Neohexene

Method

Method/ guideline followed: Calculated values using AOPWIN version 1.91, a subroutine of the computer program EIPWIN version 3.11 which uses a program described by Meylan and Howard (1993). Program used structure for neohexene

Type: air
GLP: Not applicable
Year: Not applicable

Results

Indirect photolysis

Sensitiser *(type):* OH
Rate Constant: 26.8018 E-12 cm^3/molecule-sec
Degradation % after: 50% after 4.8 hrs (using 12-hr day and avg. OH conc. of 1.5 E OH/cm^3)

Sensitiser *(type):* Ozone
Rate Constant: 0.175 E-17 cm^3/molecule-sec
Degradation % after: 50% after 6.5 days (using avg. OH conc. of 7 E11 mol/cm^3)

Reliability: (2) Reliable with restrictions. The value was calculated data based on chemical structure as modeled by EPIWIN. This robust summary has a rating of 2 because the data are calculated and not measured.

Flag: Critical study for SIDS endpoint

References:

Meylan, W.M. and Howard, P.H. 1993. Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. Chemosphere 26: 2293-99

EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

B. Stability in Water

Test Substance

Identity: CAS No. 25264-93-1, Hexene ; CAS No. 68526-52-3; Alkenes, C6 ; or CAS No. 558-37-2, Neohexene

Method

Method/ guideline followed: Other – Technical Discussion
Type *(test type):*
GLP: Yes [ ] No[ ]
Year:

Test Conditions: Not applicable

Results: Not applicable

Remarks: Hydrolysis of an organic molecule occurs when a molecule (R-X) reacts with water (H_2O) to form a new carbon-oxygen bond after the carbon-X bond is cleaved (Gould, 1959; Harris, 1982b). Mechanistically, this reaction is referred to as a nucleophilic substitution reaction, where X is the leaving group being replaced by the incoming nucleophilic oxygen from the water molecule.

The leaving group, X, must be a molecule other than carbon because for hydrolysis to occur, the R-X bond cannot be a carbon-carbon bond. The carbon atom lacks sufficient electronegativity to be a good leaving group and carbon-carbon bonds are too stable (high bond energy) to be cleaved by nucleophilic substitution. Thus, hydrocarbons, including alkenes, are not subject to hydrolysis (Harris, 1982b) and this fate process will not contribute to the degradative loss of chemical components in this category from the environment.

Under strongly acidic conditions the carbon-carbon double bond found in alkenes, such as those in the Higher Olefins Category, will react with water by an addition reaction mechanism (Gould, 1959). The reaction product is an alcohol. This reaction is not considered to be hydrolysis because the carbon-carbon linkage is not cleaved and because the reaction is freely reversible (Harris, 1982b). Substances that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Neely, 1985).

The substances in the Higher Olefins Category are primarily olefins that contain at least one double bond (alkenes). The remaining chemicals are saturated hydrocarbons (alkanes). These two groups of chemicals contain only carbon and hydrogen. As such, their molecular structure is not subject to the hydrolytic mechanism discussed above. Therefore, chemicals in the Higher Olefins Category have a very low potential to

hydrolyze, and this degradative process will not contribute to their removal in the environment.

Conclusions: In the environment, hydrolysis will not contribute to the degradation of C6 olefins.

Reliability: Not applicable

References: Gould, E.S. (1959) Mechanism and Structure in Organic Chemistry, Holt, Reinhart and Winston, New York, NY, USA.

Harris, J.C. (1982b) "Rate of Hydrolysis," Chapter 7 in: W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds., Handbook of Chemical Property Estimation Methods, McGraw-Hill Book Company, New York, NY, USA.

Neely, W. B. (1985) Hydrolysis. In: W. B. Neely and G. E. Blau, eds. Environmental Exposure from Chemicals. Vol I., pp. 157-173. CRC Press, Boca Raton, FL, USA.

C. Stability In Soil

No data available

3.2 Monitoring Data (Environment)

No data available.

3.3 Transport and Distribution

3.3.1 Transport between environmental compartments

A. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Type: Fugacity models, Mackay Levels I and III

Remarks: Trent University model used for calculations. Half-lives in water, soil and sediment estimated using EPIWIN (EPIWIN, 2000b)

Chemical assumptions:

Molecular weight:	84
Water solubility:	50 g/m^3
Vapor pressure:	23060 Pa (25°C)

Log Kow: 3.07
Melting point: -98°C
Environment name: EQC – standard environment

Half-life in air = 1.4 hr, half-life in water = 208 hr, half-life in soil = 208 hr, half-life in sediment = 832 hr

All other parameters were default values. Emissions for Level I = 1000 kg. Level III model assumed continuous 1000 kg/hr releases to each compartment (air, water and soil).

Results Media: Air, soil, water and sediment concentrations were estimated

	Level I	**Level III**
Air	100%	2.9%
Water	<1%	90.6%
Soil	<1%	5.8%
Sediment	<1%	<1%

Remarks: Since default assumptions for release estimates were used, resulting environmental concentrations are not provided.

Conclusions: These results indicated that hexene will partition primarily to air under equilibrium conditions (Level I model), but primarily to water under the assumed pattern of chemical release (equal loading of water, soil and air) in the Level III model.

Reliability: (2) Valid with restrictions: Input data are calculated.

Flag: Critical study for SIDS endpoint

References:

Trent University (2004). Level I Fugacity-based Environmental Equilibrium Partitioning Model (Version 3.00) and Level III Fugacity-based Multimedia Environmental Model (Version 2.80.1). Environmental Modeling Centre, Trent University, Peterborough, Ontario. (Available at http://www.trentu.ca/cemc)

EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

B. Test Substance

Identity: CAS No. 558-37-2, Neohexene

Method

Type: Fugacity models, Mackay Levels I and III

Remarks: Trent University model used for calculations. Half-lives in water, soil and sediment estimated using EPIWIN (EPIWIN, 2000b)

Chemical assumptions:

Molecular weight:	84
Water solubility:	32.5 g/m^3
Vapor pressure:	57460 Pa (25°C)
Log Kow:	3.04
Melting point:	-115.2°C
Environment name:	EQC – standard environment

Half-life in air = 8.52 hr, half-life in water = 360 hr, half-life in soil = 360 hr, half-life in sediment = 1440 hr

All other parameters were default values. Emissions for Level I = 1000 kg. Level III model assumed continuous 1000 kg/hr releases to each compartment (air, water and soil).

Results Media: Air, soil, water and sediment concentrations were estimated

	Level I	**Level III**
Air	100%	12.4%
Water	<1%	84.9%
Soil	<1%	1.8%
Sediment	<1%	<1%

Remarks: Since default assumptions for release estimates were used, resulting environmental concentrations are not provided.

Conclusions: These results indicated that neohexene will partition primarily to air under equilibrium conditions (Level I model), but primarily to water under the assumed pattern of chemical release (equal loading of water, soil and air) in the Level III model.

Reliability: (2) Valid with restrictions: Input data are calculated.

Flag: Critical study for SIDS endpoint

References: Trent University (2004). Level I Fugacity-based Environmental Equilibrium Partitioning Model (Version 3.00) and Level III Fugacity-based Multimedia Environmental Model (Version 2.80.1. Environmental Modeling Centre, Trent University, Peterborough, Ontario. (Available at http://www.trentu.ca/cemc)

EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

C. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Type: Volatilization from water

Remarks: Calculated using the computer program EPIWIN version 3.10; based on Henry's Law Constant of 0.383 atm-m^3/mole (calculated from VP/WS), water solubility of 50 ppm and vapor pressure of 173 mm Hg.

Results: Half-life from a model river: 0.9375 hrs
Half-life from a model lake: 3.6 days

Reliability: (2) Valid with restrictions: Some input data are calculated and values supplied by EPIWIN's experimental database were not reviewed for quality.

References: EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

D. Test Substance

Identity: CAS No. 558-37-2, Neohexene

Method

Type: Volatilization from water

Remarks: Calculated using the computer program EPIWIN version 3.11; based on Henry's Law Constant of 0.359 atm-m^3/mole (calculated by Bond SAR method).

Results: Half-life from a model river: 0.9376 hrs
Half-life from a model lake: 3.6 days

Reliability: (2) Valid with restrictions: Some input data are calculated and values supplied by EPIWIN's experimental database were not reviewed for quality.

References: EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

3.3.2 Distribution

A. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method:	Adsorption Coefficient (Koc) calculated value using the computer program EPIWIN, PCKOC v 1.66 using the method described by Meylan et al., 1992.
Test Conditions:	Based on chemical structure (program used structure for 2-hexene)

Results

Value:	Estimated Koc = 149
Reliability:	(2) Reliable with restrictions: Value is calculated.
Reference:	Meylan, W., P.H. Howard and R.S. Boethling (1992) Molecular topology/fragment contribution method for predicting soil sorption coefficients. Environ. Sci. Technol. 26:1560-7. EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

B. Test Substance

Identity:	CAS No. 558-37-2, Neohexene

Method

Method:	Adsorption Coefficient (Koc) calculated value using the computer program EPIWIN, PCKOC v 1.66 using the method described by Meylan et al., 1992.
Test Conditions:	Based on chemical structure

Results

Value:	Estimated Koc = 96.63
Reliability:	(2) Reliable with restrictions: Value is calculated.
Reference:	Meylan, W., P.H. Howard and R.S. Boethling (1992) Molecular topology/fragment contribution method for predicting soil sorption coefficients. Environ. Sci. Technol. 26:1560-7. EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

C. Test Substance

Identity:	CAS No. 25264-93-1, Hexene
Method	
Method:	Henry's Law Constant calculated value using the computer program EPIWIN, HENRY v 3.10
Test Conditions:	Bond and Group estimates based on chemical structure, at 25°C (program used structure for 2-hexene); VP/water solubility estimates based on EPIWIN values of VP = 173 mm Hg and WS = 50 mg/L.
Results	
Value:	Bond estimate = 0.423 atm-m^3/mole Group estimate = 0.370 atm-m^3/mole VP/Wsol estimate = 0.383 atm-m^3/mole
Reliability:	(2) Reliable with restrictions: Input data were from EIPWIN's database and were not reviewed for quality.
Reference:	EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

D. Test Substance

Identity:	CAS No. 558-37-2, Neohexene
Method	
Method:	Henry's Law Constant calculated value using the computer program EPIWIN, HENRY v 3.10
Test Conditions:	Bond and Group estimates based on chemical structure, at 25°C; VP/water solubility estimates based on EPIWIN values of VP = 424 mm Hg and WS = 94.4 mg/L.
Results	
Value:	Bond estimate = 0.359 atm-m^3/mole Group estimate = not estimated VP/Wsol estimate = 0.498 atm-m^3/mole
Reliability:	(2) Reliable with restrictions: Input data were from EIPWIN's database and were not reviewed for quality.
Reference:	EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

3.4 Aerobic Biodegradation

A. Test Substance

Identity: CAS No. 68526-52-3; Alkenes, C6 , internal branched

Method

Method/guideline: OECD 301F, Ready Biodegradability, Manometric Respirometry Test
Type: Aerobic [X] Anaerobic [ ]
GLP: Yes
Year: 1995
Contact time: 28 days
Inoculum: Domestic activated sludge

Test Conditions: Activated sludge and test medium were combined prior to test material addition. Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, and calcium chloride).

Test vessels were 1L glass flasks placed in a waterbath and electronically monitored for oxygen consumption.

Test material was tested in triplicate, controls and blanks were tested in duplicate. Test material loading was approximately 40 mg/L. [Reason for using 40 mg/L instead of 100 mg/L: Substances such as this test material typically have ThODs between 2 and 3 mg per mg substance. Thus, the test material concentration was adjusted for a target of 100 mg THOD/L] Sodium benzoate (positive control) concentration was approximately 44 mg/L. Test temperature was 22 +/- 1 Deg C.

All test vessels were stirred constantly for 28 days using magnetic stir bars and plates.

Results: Approximately 21% biodegradation of the test material was measured on day 28. Approximately 10% biodegradation was achieved on day 19.

By day 14, >60% biodegradation of the positive control was measured, which meets the guideline requirement. No excursions from the protocol were noted.

Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
Test Material	25.9, 10.5, 27.4	21.3
Na Benzoate	98.9,95.5	97.2

* replicate data

Reliability: (1) Reliable without restriction

Flag: Key study for SIDS endpoint

Reference: Exxon Biomedical Sciences, Inc. (1997) Alkenes, C6: Ready Biodegradability: OECD 301F Manometric Respirometry. Study #119094A. Exxon Biomedical Sciences, Inc., East Millstone, NJ, USA (unpublished report).

B. Test Substance

Identity: CAS No. 592-41-6, 1-Hexene

Method

Method/guideline: OECD 301C, Ready Biodegradability, Modified MITI Test (I)
Type: Aerobic [X] Anaerobic [ ]
GLP: no data
Year: no data
Contact time: 28 days
Inoculum: Mixture from several sources in Japan that included 4 sewage plants, 3 rivers, 2 bays, and 1 lake.

Test Conditions: A mixed inoculum was developed and maintained that used ten sources and included: return sludge from 1 industrial and 3 city sewage plants; and water from 3 rivers, 2 bays, and 1 lake, with soil from land adjacent to these bodies of water. A filtrate from the combination of these samples was prepared and added to an existing culture that had been developed from the same sources as above and maintained under aeration and with a synthetic feed composed of glucose, peptone, and monopotassium phosphate. The inoculum used for this biodegradation test was removed from the mixed culture and added to the test systems at a concentration of 30 mg of inoculum per liter of test medium. Blank and positive controls were used per guideline. The positive control, aniline, was added to the control vessel at a loading rate of 100 mg/L. Test systems contained 100 mg test substance per liter of medium. The volume of test solution was 300 ml. Temperature of incubation: 24 - 26°C. Oxygen consumption was monitored using a closed system oxygen consumption measuring apparatus from Ohkura Electric Co., Ltd. Percent biodegradation was calculated as a percent ratio of the biological oxygen demand (BOD) in the test system less the BOD of the blank control, to the calculated theoretical oxygen demand of the added test material. When percentage biodegradations of aniline calculated by BOD value were beyond 40% and 60% at the 7th and 14th day, respectively, it was concluded that the test condition was valid.

Results: The degree of biodegradation of the test material was 66 – 98% after 28 days.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
Test Material	66, 98, 67	77

* replicate data

By day 14, >60% biodegradation of the positive control was measured, which meets the guideline requirement. No excursions from the protocol were noted.

Reliability: (2) Reliable with restrictions

This study is considered valid with restrictions. Reference compound data are not presented and the range in biodegradation values is not less than 20% as required in OECD guideline 301C.

Flag: Key study for SIDS endpoint

Reference: Chemicals Inspection and Testing Institute, Japan (1992) 1-Hexene: Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Japan Chemical Industry Ecology-Toxicology and Information Center.

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

C. Test Substance

Identity: CAS No. 592-41-6; 1-Hexene

Method

Method/guideline: Closed-bottle test 84/449/EEC
Type: Aerobic [X] Anaerobic [ ]
GLP: Yes
Year: 1985
Contact time: 28 days
Inoculum: Activated sludge

Test Conditions: Microorganisms were obtained from Sittingbourne Sewage Works (UK) and prepared according to standard test protocols. Test medium was 2 mg/L 1-hexene as emulsion in Dobane PT sulphonate solution. Test bottles were incubated at 21±1°C and the extent of biodegradation was determined by measuring oxygen concentration in the bottles at days 5, 15 and 28. Controls with no microbial innoculum (control) and with medium plus microbial innoculum only (blank) were included. Sodium benzoate was used as a biodegradable substance to demonstrate the activity of the microbial innoculum.

Results: Approximately 22% biodegradation of the test material was measured on day 28. A larger amount of the theoretical oxygen demand (45%) had been consumed in the bottles titrated at day 15, but this was not sufficient for the material to pass as "readily biodegradable." There was no inhibition of microbial activity under the test conditions.

By day 15, >60% biodegradation of the positive control was measured, which meets the guideline requirement.

1-Hexene was not "readily biodegradable."

Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test material as calculated using the formula and molecular weight as C6H12=84 which leads to a Theoretical Oxygen demand of 3.43 g O per g substance and Theoretical Carbon Dioxide evolution of 3.14 g CO2 per g of test substance.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
Test Material	21, 22	22
Na Benzoate	87, 63	75

* replicate data

Reliability: (1) Reliable without restriction

Reference: Shell Research Limited (1985) Shop C6 Alpha Olefins: An Assessment of Ready Biodegradability, Document SBGR.85.111 (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

D. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method/guideline: Estimated using the computer program EPIWIN v 3.10, BIOWIN v 4.00
Type: Aerobic

Test Conditions: Estimates use methods described by Howard et al., 1992; Boethling et al., 1994; and Tunkel et al., 2000. Estimates are based upon fragment constants that were developed using multiple linear and non-linear regression analyses.

Results: Linear model prediction: Biodegrades fast
Non-linear model prediction: Biodegrades fast
Ultimate biodegradation timeframe: Days-Weeks
Primary biodegradation timeframe: Days

MITI linear model prediction: Biodegrades fast
MITI non-linear model prediction: Biodegrades fast

Reliability: (2) Reliable with restriction: Results are estimated

Reference: Boethling, R.S., P.H. Howard, W. Meylan, W. Stiteler, J. Beaumann and N. Tirado (1994) Group contribution method for predicting probability and rate of aerobic biodegradation. Environ. Sci. Technol. 28:459-65.

Howard, P.H., R.S. Boethling, W.M. Stiteler, W.M. Meylan, A.E. Hueber, J.A. Beauman and M.E. Larosche (1992) Predictive model for aerobic biodegradability developed from a file of evaluated biodegradation data. Environ. Toxicol. Chem. 11:593-603.

Tunkel, J. P.H. Howard, R.S. Boethling, W. Stiteler and H. Loonen (2000) Predicting ready biodegradability in the MITI Test. Environ. Toxicol. Chem. (accepted for publication)

EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

3.5 BOD5, COD or ratio BOD5/COD

No data available

3.6 Bioaccumulation

A. Test Substance

Identity: CAS No. 25264-93-1, Hexene

Method

Method: BCF calculated value using the computer program EPIWIN, BCF v 2.14

Test Conditions: Based on chemical structure and Log Kow (estimated as 3.07 by EPIWIN using structure for 2-hexene) using methods described by Meylan et al., 1999.

Results

Value: Estimated Log BCF = 1.666 (BCF = 46.37)

Reliability: (2) Reliable with restrictions: Input data was calculated.

Reference: Meylan,WM, Howard,PH, Boethling,RS et al. (1999) Improved method for estimating bioconcentration / bioaccumulation factor from octanol/water partition coefficient. Environ. Toxicol. Chem. 18(4): 664-672

EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

B. Test Substance

Identity: CAS No. 558-37-2, Neohexene

Method

Method: BCF calculated value using the computer program EPIWIN, BCF v 2.15

Test Conditions: Based on chemical structure and Log Kow (estimated as 3.04 by EPIWIN) using methods described by Meylan et al., 1999. Formula used to make BCF estimate: Log BCF = 0.77 log Kow – 0.70 with no correction factor.

Results

Value: Estimated Log BCF = 1.641 (BCF = 43.77)

Reliability: (2) Reliable with restrictions: Input data was calculated.

Reference: Meylan,WM, Howard,PH, Boethling,RS et al. (1999) Improved method for estimating bioconcentration / bioaccumulation factor from octanol/water partition coefficient. Environ. Toxicol. Chem. 18(4): 664-672

EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

3.7 Additional Information

A. Sewage Treatment

Test Substance

Identity: CAS No. 25264-93-1, Hexene

Test Method: Calculated, EPIWIN STP Fugacity Model, predicted fate in a wastewater treatment facility.

Input values: MW = 84.16; WS = 50 mg/L; VP = 173 mmHg; Henry's LC = 0.383148 atm-m^3/mol; air-water partition coefficient = 15.6696; Log Kow = 3.07; biomass to water partition coefficient = 235.779; temperature = 25°C

GLP: No

Test Medium: Secondary waste water treatment (water)

Test Type: Aerobic

Test Results: 99.34 % removed from wastewater treatment

Reference: EPIWIN (2000a) Estimation Program Interface for Windows, version 3.10. Syracuse Research Corporation, Syracuse, NY. USA.

B. Sewage Treatment

Test Substance

Identity: CAS No. 558-37-2, Neohexene

Test Method: Calculated, EPIWIN STP Fugacity Model, predicted fate in a wastewater treatment facility.

Input values: MW = 84.16; WS = 50 mg/L; VP = 173 mmHg; Henry's LC = 0.359 atm-m^3/mol; air-water partition coefficient = 14.682; Log Kow = 3.04; biomass to water partition coefficient = 220; temperature = 25°C

GLP: No

Test Medium: Secondary waste water treatment (water)

Test Type: Aerobic

Test Results: 99.30 % removed from wastewater treatment

Reliability: (2) Reliable with restriction: Results are estimated

Reference: EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.*

C. Sewage Treatment

Test Substance

Identity: CAS No. 592-41-6, 1-Hexene

Test Medium: Waste Water Treatment with a rotary disk contact aerator

Results: Elimination of >99% of 1-hexene.

Reliability: (2) Reliable with restrictions: Data were not reviewed for qualtity.

Reference: Verschueren, K. (1983) Handbook of Environmental Data on Organic Chemicals, 2nd ed., Van Nostrand Reinhold Company, New York, NY.

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

D. Other Information: Migration to Groundwater

Test Substance

Identity: CAS No. 592-41-6, 1-Hexene

Method: Predicted using USEPA EPIWIN Input values: MW=84.16; MP=-139.7C; BP=63.4C; WS=50 mg/L; VP=184 mmHg

Results: Rate moderate to rapid

Remarks: May be mitigated by volatilization

Reference: EAB-IRER (1995); USEPA EPIWIN output run by USEPA/OPPT/RAD/ECAB, 8/99.

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

4. ENVIRONMENTAL TOXICITY

4.1 Acute Toxicity to Fish

A. Test Substance

Identity: CAS No. 68526-52-3; Alkenes, C6

Method

Method/guideline: OECD 203
Test type: Semi-static Fish Acute Toxicity Test
GLP: Yes [X] No [ ]
Year: 1995
Species/Strain/Supplier: Rainbow Trout (Oncorhynchus mykiss)

Analytical Monitoring: Yes
Exposure period: 96 hours
Statistical methods: Trimmed Spearman-Karber Method (Hamilton, M.A. et al. 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentration in Toxicity Bioassays. Environ. Sci. Technol. 11:714-719.)

Test Conditions: Each test solution was prepared by adding the test substance, via syringe, to 19.5 L of laboratory blend water in 20 L glass carboys. The solutions were mixed for 24 hours with a vortex of $\leq$10%. Mixing was performed

using a magnetic stir plate and Teflon® coated stir bar at room temperature (approximately 22C). After mixing, the solutions were allowed to settle for one hour after which the Water Accommodated Fraction (WAF) was siphoned from the bottom of the mixing vessel through a siphon that was placed in the carboy prior to adding the test material. Test vessels were 4.0 L aspirator bottles that contained approximately 4.5 L of test solution. Each vessel was sealed with no headspace after 5 fish were added. Three replicates of each test material loading were prepared. Approximately 80% of each solution was renewed daily from a freshly prepared WAF.

Test material loading levels included: 6.25, 12.5, 25, 50, and 100 mg/L, which measured 2.9, 6.6, 13.4, 16.9, and 44.0 mg/L, respectively, and are based on the mean of samples taken from the new and old test solutions. A control containing no test material was included and the analytical results were below the quantitation limit, which was 0.2 mg/L.

Water hardness was 190-210 mg/L as CaCO3. Test temperature was 16C (sd = 0.04). Lighting was 623 to 629 Lux with a 16-hr light and 8-hr dark cycle. Dissolved oxygen ranged from 7.7 to 9.6 mg/L for "new" solutions and 4.5 to 7.5 mg/L for "old" solutions. The pH ranged from 8.2 to 8.5 for "new" solutions and 7.2 to 7.7 for "old" solutions.

Fish supplied by Thomas Fish Co. Anderson, CA, USA; age at test initiation = approximately 5 weeks; mean wt. at test termination = 0.375 g; mean total length at test termination = 3.6 cm; test loading = 0.42 g of fish/L. The fish were slightly shorter than the guideline suggestion of 4.0 to 6.0 cm, which were purposely selected to help maintain oxygen levels in the closed system. Fish size had no significant effect on study outcome.

Results:

96-hour LL50 = 12.8 mg/L (95% CI 10.7 to 15.3 mg/L) based upon loading rates.

96-hour LC50 = 6.6 mg/L (95% CI 5.4 to 8.0 mg/L) based upon measured values of old and new solutions.

Analytical method used was Headspace Gas Chromatography with Flame Ionization Detection (GC-FID).

Loading Rate (mg/L)	Measured Conc. (mg/L)	Fish Total Mortality (@96 hrs)*
Control	Control	0
6.25	2.9	0
12.5	6.6	7
25	13.4	15
50	16.9	15
100	44.0	15

* 15 fish added at test initiation

Reliability: (1) Reliable without restriction

Flag: Key study for SIDS endpoint

References Exxon Biomedical Sciences, Inc. (1996) Alkenes, C6: Fish, Acute Toxicity Test. Study #119058. Exxon Biomedical Sciences, Inc., East Millstone, NJ, USA (unpublished report).

B. Test Substance

Identity: CAS No. 592-41-6, 1-Hexene

Method

Method/guideline: 96 hour semi-static toxicity test; OECD 203; ECC C1
Test type: semi-static
GLP: Yes [X] No [ ]
Year: 1991
Species/Strain/Supplier: *Oncorhynchus mykiss*
Analytical Monitoring: Yes
Exposure period: 96 hr
Statistical methods: No data

Test Conditions: Minimal headspace to prevent losses through evaporation

Results: The mean measured concentration of 1-hexene in the test media was approx 20 to 40% that of nominal concentrations over the first 24 hours of the test but increased to 47 to 120% for the remaining exposure periods. Based upon mean measured concentrations of 1-hexene in the test media the LC_{50} value was calculated to be:

$LC_{50}(24h) = 9.7$ mg/L
$LC_{50}(48h) = 5.6$ mg/L
$LC_{50}(72h) = 5.6$ mg/L
$LC_{50}(96h) = 5.6$ mg/L

Reliability: (2) Reliable with restrictions: Reliable laboratory but minimal information about methods was available.

Flag: Key study for SIDS endpoint

References: Shell Research Limited (1991) 1-Hexene: Acute Toxicity to Oncorhynchus mykiss, SBGR.91.252 (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11.

4.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

A. Test Substance[MM1]

Identity:	CAS No. 68526-52-3, Alkenes C6
Remarks:	Composition: C5 n-olefins = 0.5%, C5 iso-olefins = 1.3%, C6 n-olefins = 10.4%, C6 iso-olefins = 55.6%, C5 n-paraffins = 3.3%, C5 iso-paraffins = 9.3%, C6 iso-paraffins = 17.8%, C7 iso-olefins = 1.0%

Method

Method/guideline followed:	OECD Guideline 202, Acute Toxicity to water Fleas, (*Daphnia magna*) Under Static and Sealed Vessel Conditions
Test type:	Static
GLP:	Yes
Year:	2003
Analytical Monitoring:	Yes
Species/Strain:	*Daphnia magna*
Exposure period:	48 hr
Statistical methods:	If at least one concentration caused immobilization of ≥ 50% of the test population, a computer program (TOXSTAT Version 3.5) was used to estimate EC50 and EL50 values using several statistical methods (e.g., probit, Spearman-Karber)

Test Conditions

TEST ORGANISMS

- Source/supplier: Obtained from laboratory cultures maintained at Springborn Smithers Laboratory
- Feeding: *Ankistrodesmus falcatus* (4 x 10^7cells/mL), 2.0 mL per vessel/day, in addition to a 0.5 mL suspension of yeast, cereal leaves and flaked fish food.
- Age at study initiation: Approximately 24 hr old
- Control: A test conducted on 11 February 2003 established that the 24-hr LC50 for potassium dichromate was between 0.10 and 10 mg/L.

STOCK AND TEST SOLUTION AND THEIR PREPARATION

- A water accommodated fraction (WAF) of each loading rate was prepared at test initiation by spiking the appropriate volume of test substance corrected for a density of 0.6809 g/ml, via gas-tight syringe, directly into dilution water in a 2-L Mariotte bottle. The bottle was filled to the neck (approx. 1% volume headspace) and the test substance was spiked below the water/air interface and capped immediately with a silicone stopper wrapped in aluminum foil. The solutions were placed on a magnetic stir plate and stirred for approximately 24 hr with minimal vortex. The WAF was maintained at room temperature and covered with aluminum foil. Following stirring, the contents were allowed to settle for approximately 15 min before the WAF was removed, slowly from the lower side-wall drain, directly into each exposure vessel after first discarding the initial aliquot. Care was taken to reduce volatilization during handling. A control solution was prepared following the same procedures except without the addition of test substance.

DILUTION WATER
- Source: Well water fortified based on the formula for hard water (U.S. EPA, 1975) and filtering it through an Amberlite XAD-7 resin column to remove any potential organic contaminants
- Hardness: 180 mg/L as CaCO3
- Alkalinity: 110 mg/L as CaCO3
- pH: 7.9
-Specific conductivity: 500 micromhos per centimeter
- Analysis for contaminants: Periodically analyzed to ensure that pesticides, PCBs and toxic metals are not present at concentrations that are considered toxic.
-TOC concentration: Water sampled during the month of the study was found to have a total organic carbon concentration of 1.2 mg/L
TEST SYSTEM
- Temperature: 20-21°C
- Dissolved oxygen concentration: 7.7 – 8.4 mg/L
- pH: 7.8 – 8.0
- Renewal of test solution: No
- Exposure vessel type: 250 mL test solution in a 250-mL glass Erlenmeyer flask with Teflon®-lined screw caps; minimal headspace
- Number of replicates/individuals per replicate: 5 / 5
- Intensity of irradiation: 60-80 footcandles
- Photoperiod: 16h:8h light-dark cycle
TEST PARAMETER: Immobilization relative to the control (defined as inability to swim within 15 seconds after gentle agitation of test container
MONITORING OF TEST SUBSTANCE CONCENTRATION: Measured by HPLC/UV at test initiation and termination
DEVIATIONS FROM GUIDELINE: The guideline states that the daphnids will be <24 hr old at initiation of the test. The maximum age of the daphnids in this study was 24.5 hrs. Most daphnids were less than 24 hr old. The 2 range-finding studies used daphnids less than 24 hrs old and those results corroborated the results of the definitive test, demonstrating that the deviation did not impact the sensitivity of the definitive exposure.

Results

Nominal conc.:	6.7, 14, 27, 54, 110 mg/L (nominal loading rates)
Measured conc.:	0.34, 2.4, 6.7, 12 and 19 mg/L
EL50:	20 mg/L at 48 hrs (using nominal loading rates)
EC50:	4.4 mg/L at 48 hrs (using geometric mean measured concentrations)
Remarks:	PRELIMINARY TEST: Daphnids were exposed under static conditions to WAFs at nominal loading rates of 0.50, 2.0, 5.1 and 20 mg/L. At 48 hrs, no immobilization or adverse effects were observed in any of the loading rates or the control. At test initiation and test termination, measured concentrations of the lowest loading rate were below detectable limits, and measured concentrations of the highest loading rate at test initiation and termination were 2.2 and 0.82 mg/L respectively.

Based on these results, further exploratory work was conducted to determine the optimal procedures and loading rates for the definitive study.

DEFINITIVE TEST: Following 48 hrs of exposure, 100% immobilization was observed among daphnids exposed to the 3 highest treatments (nominal loading rates of 27, 54 and 110 mg/L). Immobilization of 5% was observed at 14 mg/L nominal loading rate. Adverse effects (e.g., lethargy, on the bottom of the test vessel) were observed in all mobile daphnids exposed to this concentration. No immobilization or adverse effects were observed at the nominal loading rate of 6.7 mg/L or the control.

Reliability: (1) Reliable without restrictions

Flag: Key study for SIDS endpoint

References Hoberg, J.R. (2003) C6 Hexene – Acute Toxicity Test with the Water Fleas, *Daphnia magna*, Under Static and Sealed Vessel Conditions. Study No. 13761.6114, Springborn Smithers Laboratories, Wareham, Massachusetts, conducted for American Chemistry Council (Higher Olefins Panel) (unpublished report).

B. Test Substance

Identity: CAS No. 592-41-6, 1-Hexene (approx. 99%)

Method

Method/guideline:	48-hr static toxicity test
Test type:	Static
GLP:	Yes [ ] No [X]
Year::	1985
Analytical Monitoring:	No
Species/Strain:	*Daphnia magna*
Exposure period:	48 hrs
Statistical methods:	The LC50 values were calculated by means of a parametric model developed by Kooijman [Kooijman, S.A.L.M. (1981) Parametric analyses of mortality rates in bioassays. Water Res. 15:105-119.]

Test Conditions: Test media were prepared by adding test material to 500 ml of fresh water (pH ~8, hardness ~210 mg $CaCO_3$ per liter) and stirring for 4 hr before adding test animals (25/glass beaker covered with a watch glass or glass-stoppered conical flask; 20 °C; no aeration, food, replicate or media renewal). The test animals were less than 24 h old at the start of the test. At none of the dose levels was test substance visible during the test period. pH and oxygen were monitored. During the test, oxygen concentration was >70% of saturation level.

Results:	Nominal concentrations were 0, 3.2, 10, 32, 100 mg/l. 48 hr EL50 for beaker covered with watch glass = (est.) 60 mg/l; NOEC after 48 hr = 32 mg/l (nominal) 48 hr EL50 for stoppered flask = (est.) 30 mg/l; NOEC after 48 hr = 10 mg/l (nominal)
Reliability	(2) Reliable with restrictions. Study does not totally comply with current testing guidelines. No chemical analyses were performed.
References:	Adema, D.M.M. and Bakker, G.H. (1985) Aquatic toxicity of compounds that may be carried by ships [MARPOL 1973; Annex II]. TNO report R 85/217, The Hague (unpublished report).
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

4.3 Toxicity to Aquatic Plants (e.g. Algae)

A. Test Substance[MM2]

Identity:	CAS No. 68526-52-3, Alkenes C6
Remarks:	C5 n-olefins = 0.5%, C5 iso-olefins = 1.3%, C6 n-olefins = 10.4%, C6 iso-olefins = 55.6%, C5 n-paraffins = 3.3%, C5 iso-paraffins = 9.3%, C6 iso-paraffins = 17.8%, C7 iso-olefins = 1.0%

Method

Method/guideline:	OECD 201, Alga, Growth Inhibition Test
Test type:	static
GLP:	Yes [X] No [ ]
Year:	2003
Analytical Monitoring:	yes
Species/Strain:	*Pseudokirchneriella subcapitata*
Element basis:	Number of cells/mL, area under the curve, growth rate
Exposure period:	96 hrs
Statistical methods:	Cell density was calculated by dividing the number of cells counted by the number of fields examined. Growth rate and biomass (area under the growth curve) for each replicate vessel were calculated using formulas recommended in the OECD 201 testing guideline. EC values and their 95% confidence limits were determined by linear regression of response versus exposure concentration expressed as nominal loading rate and geometric mean measured concentration over the range of test concentrations, using the computer program, Toxstat (Gulley, D.D., A.M. Boetler, and H.L. Bergman (1966) Toxstat Release 3.5. University of Wyoming, Laramie, Wyoming). To determine the NOEC, the data were first checked for normality using Shapiro-Wilks' Test and, for

homogeneity of variance using Bartlett's Test. If the data sets passed the test for homogeneity and normality, William's Test (Williams, D.A. [1971] A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27:103-117; [1972] A comparison of several dose levels with a zero control. Biometrics 28:519-531) was used to determine the NOEC. All statistical determinations were made at the 95% level of certainty, except in the case of Shapiro-Wilks' and Bartlett's Tests, where the 99% level of certainty was applied.

Test Conditions :

TEST ORGANISMS
- Strain: 1648 obtained from the University of Texas, Austin, TX, USA.
- Initial cell concentration: 1.0 x 10^4 cells/mL

STOCK AND TEST SOLUTION AND THEIR PREPARATION
- A water accommodated fraction (WAF) of each loading rate was prepared 1 day prior to test initiation by adding the appropriate weight of test substance via gas-tight syringe, directly into the test medium containing an additional 300 mg/L of sodium bicarbonate. Each WAF was contained in a 2.2L Mariotte bottle filled to 99% total volume, capped with stoppers covered in aluminum foil, placed on a magnetic stir plate and stirred for approximately 24 hr with minimal vortex. The WAF was maintained at room temperature and covered with aluminum foil to avoid photodegradation during mixing. Following stirring, the contents were allowed to settle for approximately 15 minutes before the WAF was removed from the drain (lower side wall) directly into the exposure vessels. A control solution was prepared following the same procedures except without the addition of test substance.

GROWTH/TEST MEDIUM CHEMISTRY
- Stock solution contained (per liter): 25.5 mg NaNO3, 12.16 mg MgCl2•6H2O, 4.41 mg CaCl2•2H2O, 14.7 mg MgSO4•7H2O, 1.368 mg K2HPO4•3H2O, 15.0 mg NaHCO3, 185.5 µg H3BO3, 1.88 µg Na2SeO4, 415.4 µg MnCl2•4H2O, 3.270 µg ZnCl2, 1.43 µg CoCl2•6H2O, 0.012 µg CuCl2•2H2O, 7.26 µg Na2MoO4•2H2O, 159.8 µg FeCl3• 6H2O, 300.0 µg Na2EDTA•2H2O.

DILUTION WATER
Sterile deionized laboratory water, which is periodically analyzed to ensure that pesticides, PCBs and toxic metals are not present at concentrations that are considered toxic. Water sampled during the month of the study was found to have a total organic carbon concentration of 0.74 mg/L

TEST SYSTEM
- Exposure vessel type: 45 mL medium in 45 ml volatile organic analysis vial closed with screw cap; zero headspace
- Number of replicates: triplicate
- Nominal loading rates: 3.2, 6.9, 14, 27, 54 and 110 mg/L
- Test temperature: 23-24 °C
- pH: 8.3 at start and 9.6 – 10.0 at end of the test
- Intensity of irradiation: 590 – 890 footcandles (6400 – 9600 lux)
- Photoperiod: continuous
- Shaking: 100 rpm

Method :	MONITORING OF TEST SUBSTANCE CONCENTRATION: at test initiation; 24 and 72 hrs of exposure; and end of test (96 hr) DEVIATIONS FROM GUIDELINE: none reported ANALYTICAL METHODS: HPLC/UV

Results:

Nominal conc.:	3.4, 6.9, 14, 27, 54, and 110 mg/L (mass of test substance per water volume used in WAF preparation)
Measured conc.:	0.23, 1.8, 1.8, 6.3 3.6 and 5.5 mg/L (geometric mean)
EC50 (cell density):	84 mg/L (74-98) mg/L (96 h, nominal loading rate) 4.6 (4.3-5.0) mg/L (96 h, measured concentration)
NOEC (cell density):	14 mg/L (96 h, nominal loading rate) 1.8 mg/L (96 h, measured concentration)
E_bC50 (biomass):	92 mg/L (25-110) mg/L (72 h, nominal loading rate) 4.9 (3.8-6.2) mg/L (72 h, measured concentration) 79 mg/L (66-88) mg/L (96 h, nominal loading rate) 4.5 (4.0-4.8) mg/L (96 h, measured concentration)
NOEC (biomass):	3.4 mg/L (72 h and 96 h, nominal loading rate) 0.23 mg/L (72 h and 96 h, measured concentration)
E_rC50 (growth rate):	>110 mg/L (72 h and 96 h, nominal loading rate) >5.5 mg/L (72 h and 96 h, measured concentration)
NOEC (growth rate):	3.4 mg/L (72 h) and 14 mg/L (96 h) (nominal loading rate) 0.23 mg/L (72 h) and 1.8 mg/L (96 h) (measured concentration)
Remarks:	- Cell density data: The 96-hr cell density in the control averaged 223 x 10^4 cells/mL. Cell densities in the 3.4, 6.9, 14, 27, 54, and 110 mg/L loading rates averaged 217, 193, 205, 132, 143 and 89 x 10^4 cells/mL, respectively. - Cell biomass data: The 0- to 72-hr biomass in the control averaged 99.5 x 10^4 cells•days/mL. Biomass in the 3.4, 6.9, 14, 27, 54 and 110 mg/L loading rates averaged 104.2, 75.3, 81.8, 52.4, 58.7 and 48.8 x 10^4 cells•day/mL, respectively. The 0- to 96-hr biomass in the control averaged 270.9 x 10^4 cells•days/mL. Biomass in the 3.4, 6.9, 14, 27, 54 and 110 mg/L loading rates averaged 273.7, 210.1, 220.0,141.0, 162.1 and 117.3 x 10^4 cells•day/mL, respectively. - Growth rate data: The 0- to 72-hr growth rate in the control averaged 1.57 $days^{-1}$. The 0- to 72-hr growth rate in the 3.4, 6.9, 14, 27, 54 and 110 mg/L loading rates averaged 1.57, 1.42, 1.40, 1.26, 1.36 and 1.27 $days^{-1}$, respectively. Statistical analyses determined significant reductions in the 0- to 72-hr growth rates at $\geq$ 6.9 mg/L loading rate. The 0- to 96-hr growth rate in the control averaged 1.33 $days^{-1}$. The 0- 96-hr growth rate in the 3.4, 6.9, 14, 27, 54 and 110 mg/L loading rates averaged 1.32, 1.29, 1.31, 1.20, 1.22 and 1.10 $days^{-1}$, respectively. Statistical analyses determined significant reductions in the 0- to 96-hr growth rates at $\geq$ 27 mg/L loading rate.

Reliability: (1) Reliable without restrictions.

Flag: Key study for SIDS endpoint

References: Hoberg, J.R. (2003) C6 Hexene – Toxicity to the freshwater green alga, *Pseudokirchneriella subcapitata*, Study No. 13761.6113, Springborn Smithers Laboratories, Wareham, Massachusetts, conducted for American Chemistry Council (Higher Olefins Panel) (unpublished report).

B. Test Substance

Identity: CAS No. 592-41-6, 1-Hexene (Shop C6 Linear Alpha Olefin, >96% purity)

Method

Method/guideline: 4-day growth experiment
Test type: static
GLP: Yes [X] No [ ]
Year: 1985
Analytical Monitoring:
Species/Strain: *Selenastrum capricornutum*
Element basis:
Exposure period: 96 hrs
Statistical methods:

Test Conditions : *S.capricornutum* was taken from the axenic laboratory culture. The culture is derived from a strain (ATCC22662) obtained from the American Type Culture Collection, Maryland, USA. Sixteen Erlenmeyer flasks containing 50 ml of culture medium were prepared. To ten of those were added quantities of stock solutions of the 1-hexene in Analar acetone to give an approximately logarithmically spaced series of concentrations ranging from 1.0 to 1000 mg/L . The remaining six flasks received no olefin and served as controls. The concentration of acetone in all test flasks, including the controls, was adjusted to 0.1 ml/L. Each flask was inoculated with sufficient *S.capricornutum* to give an initial concentration of 500 cells. The flasks were incubated in a cooled, orbital incubator (100 cycles min) under constant illumination (3000 lux) at 22-26^0 C for 4 days. After 2 and 4 days incubation cell counts were made using a Coulter counter. The pH of the test solutions during the test was 7.1-7.5.

Results: EC50 (96h) = >1000 mg/L (nominal concentration) (author assigned)
EC50 (96h) = > solubility
EL0 (96h) = >22 mg/L (see remarks)

Remarks: The highest nominal concentration of 1-hexene tested, 1000 mg/L, did not cause reduction in cell numbers at day 4 compared to the mean cell number at day 4 in the controls.

Upon review of the study, it was noted that five concentrations were tested above the water solubility of Shop C6 Alpha Olefin (1-hexene). In light of those concentrations being above the water solubility it was determined to assign the ELO (96h) with a value of > 22 mg/L. 22 mg/L was the highest nominal concentration below the water solubility at which the substance was tested. ELO = effect loading based on WAF testing procedure; no effect observed at the highest loading indicated.

Reliability: (2) Reliable with restrictions. Study does not totally comply with current testing guidelines. No chemical analyses were performed.

References: Shell Research Limited (1985) Shop C6 Linear Alpha Olefins (1-Hexene); Acute Toxicity (Salmo gairdneri, Daphnia magna and Selenastrum capricornutum) and N-Octanol/Water Partition Coefficient, SBGR.85.026 (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

4.4 Toxicity to Micro-organisms, e.g. Bacteria

A. Test Substance

Identity: CAS No. 592-41-6, 1-Hexene

Method

Method: According to 79/931/EEC, Annex V "Degradability, Ecotoxicity, and Bioaccumulation, TNO, Delft, The Netherlands, 1977.
GLP: Yes
Species: *Pseudomonas fluorescens*
Exposure Period: 6 hr
Analytical Monitoring: No data

Conditions: Test substance was dissolved in ethanol to give a stock solution containing 500g/l of SHOP C6 linear alpha olefin. Dilutions of this solution in test medium were made such that the final concentrations of SHOP C6 in the test were 1000, 320, 100, 32, and 10 mg/l. Sodium

pentachlorophenate was used as a standard inhibitory substance. Controls containing the microbial inoculum and no inhibitory substances were used to assess the logarithmic growth rate of the organisms under non-inhibitory conditions. Growth curves were constructed of the optical density of the inoculated media versus time and the rate determined as the slope of the exponential growth phase.

$$\% \text{ inhibition} = \frac{\text{growth rate control- growth rate test}}{\text{growth rate control}} \times 100$$

Results: Maximum inhibition was 24% at 1000 mg/L

Remarks: The standard substance, sodium pentachlorophenate, inhibited with an EC50=30 mg/l while the test substance SHOP C6 caused a maximum inhibition of 24% at 1000 mg/l.

Reliability: (1) Reliable without restrictions.

Reference: Shell Research Limited (1985) Shop C6 Alpha Olefins: An Assessment of Ready Biodegradability, Document SBGR.85.111 (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11.

B. Test Substance

Identity: CAS No. 592-41-6, 1-Hexene; CAS No. 111-66-0, 1-Octene; CAS No. 872-05-9, 1-Decene; CAS No. 1120-36-1, 1-Tetradecene (Analytical Grade)

Method

Method: Acute static bioassay
GLP: No
Type: Aquatic
Species: Thirteen marine bacteria
Exposure Period: 16 hours
Analytical Monitoring: No data

Test Conditions: Water samples collected from Cleveland and Victoria Point on the Brisbane coast, southeastern Queensland, Australia, were cultured on marine salts medium solidified with 1.5% agar. Thirteen different marine bacteria were isolated and transferred to new media. This culture was maintained at 30°C and subcultured weekly. The test articles were dissolved in ethanol and added to media (maximum 0.1 ml in 50 ml). 0.1 mg of bacterial culture containing 8×10^{10} bacteria per ml was added. Each experiment was performed in triplicate. Controls consisting of bacteria inoculated into the medium, without test compounds, both with and without ethanol were run simultaneously. Absorbance at 600 nm was determined, followed by incubation without shaking at 30°C. After 16

hours, the absorbance was remeasured and the differences were calculated and expressed as a percentage of the difference in absorbance of the control. These data were then converted to Probit units and least-squares linear regression equation against toxicant concentration was obtained. From these regression equations, the effective concentration of the test compound that inhibits bacterial growth by 50 and /or 10% (EC50 and EC10, respectively) was determined.

Results: Only 1-hexene exerted a toxic effect [log EC10 = -0.49]; however, the calculated log EC50 was 0.46, indicating a value >100% saturation in sea water. The other 1-alkenes were not toxic up to levels of 100% saturation.

Reliability: (1) Reliable without restrictions

Reference: Warne, M. St. J. Connell, D.W., Hawker, D. W., and G. Schuurmann (1989) Quantitative Structure-Activity Relationships for the Toxicity of Selected Shale Oil Components to Mixed Marine Bacteria. Ecotoxicology and Environmental Safety, 17: 133-148.

Other: This study was included in the dossiers for 1-hexene and 1-tetradecene at SIAM 11. Additional information has been added.

4.5 Chronic Toxicity to Aquatic Organisms

A. Chronic Toxicity to Fish

Test Substance: CAS No. 25264-93-1, Hexene ; CAS No. 68526-52-3, Alkenes, C6; or CAS No. 558-37-2, Neohexene

Method/Guideline:

Type (test type): 30-day Chronic Toxicity Value (ChV) calculated using the computer program ECOSAR, version 0.99g included in the EPI Suite software, v 3.11 (EPIWIN, 2000b)

Species: Fish

Test Conditions: The program uses structure-activity relationships (SARs) to predict the aquatic toxicity of chemicals based on their similarity of structure to chemicals for which the aquatic toxicity has been previously measured. The program uses regression equations developed for chemical classes using the measured aquatic toxicity values and estimated Kow values. Toxicity values for new chemicals are calculated by inserting the estimated Kow into the regression equation and correcting the resultant value for the molecular weight of the compound. The CAS number was used for input into EPIWIN. The program used Kow values of 3.15 for hexene; 3.07 for Alkenes, C6; and 3.04 for neohexene. The Kow values were estimated by EPIWIN using the structure for 1-hexene for hexene;

and the structure for 2-hexene for alkenes, C6 ; and a structure with branching for neohexene.

Results:

Units/Value: Estimated 30-day ChV for hexene = 942 µg/L
Estimated 30-day ChV for Alkenes, C6 = 803 µg/L
Estimated 30-day ChV for neohexene = 1001 µg/L

Flag: Key study for SIDS endpoint

Reliability: (2) Reliable with restrictions. The result is calculated data.

Reference: EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

B. Chronic Toxicity to Aquatic Invertebrates

Test Substance: CAS No. 25264-93-1, hexene; CAS No. 68526-52-3, Alkenes, C6 ; CAS No. 558-37-2, Neohexene

Method/Guideline:

Type (test type): 16-day EC50 value calculated using the computer program ECOSAR, version 0.99g included in the EPI Suite software, v 3.11 (EPIWIN, 2000b)

Species: ***Daphnia magna***

Test Conditions: The program uses structure-activity relationships (SARs) to predict the aquatic toxicity of chemicals based on their similarity of structure to chemicals for which the aquatic toxicity has been previously measured. The program uses regression equations developed for chemical classes using the measured aquatic toxicity values and estimated Kow values. Toxicity values for new chemicals are calculated by inserting the estimated Kow into the regression equation and correcting the resultant value for the molecular weight of the compound. The CAS number was used for input into EPIWIN. The program used Kow values of 3.15 for hexene; 3.07 for Alkenes, C6; and 3.04 for neohexene. The Kow values were estimated by EPIWIN using the structure for 1-hexene for hexene; and the structure for 2-hexene for alkenes, C6 ; and a structure with branching for neohexene.

Results:

Units/Value: Estimated 16-day EC50 for hexene = 582 µg/L
Estimated 16-day EC50 for Alkenes, C6 = 509 µg/L
Estimated 16-day EC50 for neohexene = 611 µg/L

Flag: Key study for SIDS endpoint

Reliability: (2) Reliable with restrictions. The result is calculated data.

Reference: EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

4.6 Toxicity to Terrestrial Organisms

A. Toxicity to Terrestrial Plants.

Test Substance: CAS No. 25264-93-1, hexene; CAS No. 68526-52-3, Alkenes, C6 ; CAS No. 558-37-2, Neohexene

Method/Guideline:

Type (test type): 96-hr Chronic Toxicity Value (ChV) calculated using the computer program ECOSAR, version 0.99g included in the EPI Suite software, v 3.11 (EPIWIN, 2000b)

Species: Green algae

Test Conditions: The program uses structure-activity relationships (SARs) to predict the aquatic toxicity of chemicals based on their similarity of structure to chemicals for which the aquatic toxicity has been previously measured. The program uses regression equations developed for chemical classes using the measured aquatic toxicity values and estimated Kow values. Toxicity values for new chemicals are calculated by inserting the estimated Kow into the regression equation and correcting the resultant value for the molecular weight of the compound. The CAS number was used for input into EPIWIN. The program used Kow values of 3.15 for hexene; 3.07 for Alkenes, C6; and 3.04 for neohexene. The Kow values were estimated by EPIWIN using the structure for 1-hexene for hexene; and the structure for 2-hexene for alkenes, C6 ; and a structure with branching for neohexene.

Results:

Units/Value: Estimated 96-hr ChV for hexene = 876 µg/L
Estimated 96-hr ChV for Alkenes, C6 = 780 µg/L
Estimated 96-hr ChV for neohexene = 916 µg/L

Flag: Key study for SIDS endpoint

Reliability: (2) Reliable with restrictions. The result is calculated data.

Reference: EPIWIN (2000b). Estimation Program Interface for Windows, version 3.11. EPI Suite™ software, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, U.S.A.

B. Toxicity to Soil Dwelling Organisms.

No data available

C. Toxicity to Other Non Mammalian Terrestrial Species (including Avian)

No data available

4.7 Biological EffectsMonitoring (including Biomagnification)

No data available

4.8 Biotransformation and Kinetics

No data available

5. MAMMALIAN TOXICITY

5.1 Toxicokinetics, Metabolism and Distribution

A. Test Substance: CAS No. 592-41-6, 1-Hexene (NEODENE 6 alpha olefin)

Method	
Test Type	In-vitro
GLP	No
Year	1984

Method: Shell Protocol 61, using Biuret reagent

Test Conditions: Microsomes were prepared from livers of adult, male Fischer-344 rats, approximately 225 grams, pretreated with Phenobarbital. Incubations were carried out in sealed serum bottles. Reaction mixtures contained: microsomal protein (1mg/ml), KCl (150 mM), EDTA (1.5 mM), Na/K phosphate buffer (0.1M, pH 7.4), 1-hexene in ethanol (0.1-4mM)

Results: NEODENE 6 alpha olefin (1-hexene) was tested in an in vitro system and was demonstrated to cause the autocatalytic ("suicidal") destruction of cytochrome P-450 and heme in hepatic microsomes from phenobarbital pretreated rats. The destructive process was time dependent, saturable and required NADPH. Destruction was

inhibited by metyrapone and carbon monoxide but not by glutathione. Reduced oxygen tension did not prevent the loss of cytochrome P-450. NEODENE 6 alpha olefin was shown to be a substrate for cytochrome P-450 by its binding spectrum to the cytochrome.

Reliability: (1) Reliable without restriction

Reference: Shell Development Company (1984) In vitro Destruction of Hepatic Cytochrome P-450 and Heme by NEODENE 6 Alpha Olefin, WRC-814, (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

B. Test Substance: CAS No. 592-41-6, 1-Hexene

Method

Test Type:	In vivo
GLP	No data available
Year	1995

Method: Some olefins have been shown to be metabolized to epoxides. For example, ethylene and propylene have been shown to be metabolized to their corresponding oxides by the presence in animals of the corresponding hemoglobin and DNA adducts. Absorption, distribution, elimination and hemoglobin and DNA adduct formation were studied in the rat after inhalation of individual C_2 - C_8 1-alkenes [including 1-hexene] at 300 ppm, 12 hr /day for 3 consecutive days. Concentrations of olefins were measured in blood, lung, brain, liver, kidney and perirenal fat immediately after each exposure and 12 h after the third exposure.

Results: Concentrations of olefins reached steady state levels after the first 12 hr of exposure, and the concentrations 12 hr after the last exposure were generally low (<3% of the concentrations immediately after exposure), except in the fat. Concentrations of 1-alkenes in blood and tissues increased with increasing number of carbon atoms. In contrast, levels of hemoglobin and DNA adducts decreased with increasing number of carbon atoms. The decrease was most pronounced from C2 to C3.

Concentrations of individual 1-alkenes after the third daily 12 hr exposure to 300 ppm and concentrations in fat 12 hr after the third exposure (n=4). All concentrations are in μmol/kg; nd = not detectable (detection limits not provided)

Chemical	Blood	Liver	Lung	Brain	Kidneys	Fat	Fat 12 hr after 3rd exposure
Ethene	0.3	0.4	2.3	0.7	0.7	7	nd
Propene	1.1	0.3	2.9	1.7	1.8	36	nd
1-Butene	1.9	0.8	4.9	3.0	5.7	70	0.3
1-Pentene	8.6	51.6	31.4	41.0	105.7	368	19
1-Hexene	18.2	66.8	59.7	59.7	188.0	1031	77
1-Heptene	37.0	138.3	85.6	109.3	269.3	2598	293
1-Octene	60.1	443.7	202.4	270.0	385.1	4621	943

Remarks: The increased retention in fat of 1-alkenes with higher carbon numbers is presumably a function of their increased lipophilicity, and decreased likelihood to be exhaled unchanged, compared to the lower volatile 1-alkenes. Since unchanged 1-alkenes are not considered to be toxic, and because tissue levels rapidly cleared after exposure ceased, this concentration, especially in fat tissues, is unlikely to have any biological effect. An implication of the metabolic formation of an epoxide, as determined by hemoglobin and DNA adducts, is that the 1-alkenes are likely to be genotoxic. However ethylene, which formed these adducts to a much greater extent than the higher homologs, has been specifically investigated in lifetime animal cancer bioassays at concentrations up to 3000 ppm, and determined to be negative [Hamm, T.E. Jr., Guest, D, and Dent, J.G. (1984) Fundam. Appl. Toxicol. 4(3 Pt 1):473-8]. It is highly unlikely that the higher homologs, including 1-hexene, will be genotoxic or carcinogenic under these conditions.

Reliability: (1) Reliable without restrictions.

Reference: Eide, I., R. Hagerman, K. Zahlsen, E. Tareke, M. Tornquist, R. Kumar, P. Vodicka and K. Hemminki (1995) Uptake, distribution, and formation of hemoglobin and DNA adducts after inhalation of C2-C8 1-alkenes [olefins] in the rat. Carcinogenesis. 16, 1603 - 1609.

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

5.2 Acute Toxicity

A. Acute oral toxicity

(1) **Test Substance**

Identity (purity):	CAS No. 592-41-6, 1-Hexene (NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 401
Type *(test type):*	LD50
GLP:	Yes [X] No [ ]
Year*:*	1982
Species/Strain:	Rat/F344
Sex:	Males and females
No. of animals per sex per dose:	5
Vehicle:	
Route of administration:	oral gavage
Test Conditions:	No data

Results:

Value:	LD50 > 5.6 g/kg
Number of deaths at each dose level:	No deaths in 5 males or 5 females at 5.6 g/kg
Remarks:	No treatment-related gross pathology
Reliability:	(1) Reliable without restrictions
Flag:	Key study for SIDS endpoint.
References:	Shell Development Company (1982) Acute Oral Toxicity of NEODENE 6 Alpha Olefin in the Rat, WTP-120 (unpublished report).
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(2) **Test Substance**

Identity (purity):	CAS# 558-37-2, Neohexene (3,3-dimethylbutene-1, 98.5% purity)

Method

Method/guideline:	OECD 401
Type *(test type):*	LD50

GLP:	Not specified
Year:	1982
Species/Strain:	Rat/Sprague-Dawley
Sex:	Males and females
No. of animals per sex per dose:	5
Vehicle:	None
Route of administration:	oral gavage

Test Conditions: One group of five rats/sex was dosed orally at a level of 5000 mg/kg of body weight. At initiation of the study, the males weighed 270 to 294 g and the females 202 to 233 g The animals were observed at 1, 2, and 4 hours after dosing, and daily for a period of 14 days for mortality and signs of systemic toxicity. Body weights were recorded prior to treatment and at 7 and 14 days. The animals were necropsied at the end of the 14-day period and observed for gross abnormalities. Statistical methods were not specified.

Results:

Value:	LD50 > 5 g/kg
Number of deaths at each dose level:	No deaths in 5 males or 5 females at 5 g/kg
Remarks:	Clinical signs of toxicity noted 1 hour after dosing included depression, soft feces, a hunched appearance, and rough fur coat. All animals appeared normal from Day 2 through termination of the study. All animals gained weight during the study. There were no significant findings at necropsy.

Reliability: (1) Reliable without restrictions

References: Hazleton Laboratories America, Inc. (1982). Neohexene: Acute Oral Toxicity Study in Rats. Conducted for Phillips Petroleum Company, (unpublished report).

B. Acute inhalation toxicity

(1) Test Substance

Identity (purity): CAS No. 592-41-6, 1-Hexene

Method

Method/guideline: 4-hr exposures to seven different concentrations in a dynamic exposure system.

Type *(test type):* LC50
GLP: Yes [ ] No [X]
Year: 1967
Species/Strain: Rat/Wistar
Sex: Males
No. of animals per sex per dose: 10

Vehicle: None
Route of administration: Inhalation

Test Conditions: Groups of 10 male rats weighing between 200 and 290 g (age not specified) were exposed to various vapor concentrations of 1-hexene for 4 hours. During exposure, animals were observed for toxic signs, and fatalities were autopsied after the exposure was terminated. Survivors were observed and weighed periodically for 14 days and then sacrificed for determination of gross pathological change. Concentrations were established by preparing saturated vapors and diluting with air to the desired degree. After equilibration of the chamber, the animals were introduced. During the exposure, several air samples were withdrawn from the changer, further diluted by a known amount with air to bring the resulting concentration below the L.E.L. (Lower Explosive Limit), and passed through an M.S.A. Combustible Gas Indicator which had been calibrated for 1-hexene. This analytically derived chamber concentration was compared with the theoretical chamber concentration based on weight loss of the bubbler and the total amount of air passing through the chamber for the given operating situation. The acute vapor toxicity for a 4- hr exposure was estimated by plotting the percentage mortality vs chamber concentration and applying the Litchfield method to obtain the LC50.

Results:

Value: LC50: 32,000 ppm (110 mg/L)
Number of deaths at each dose level: See table below.

Remarks: No treatment-related gross pathology

Concentration (ppm):	0	27600	28600	30500	33200	37000	41200
Mortality	0/10	0/10	2/10	5/10	7/10	8/10	10/10
Onset of anesthesia, min	None	15	20	20	10	10	5

Time range of death, min	None	>240	120-240	90-240	60-240	60-235	40-235
Bodyweight changes at Day 14	+55%	+50%	+48%	+50%	+55%	+45%	--

Reported vapor concentrations were nominal values based on the total airflow and the weight loss of the vapor generator. Vapor concentration values obtained with a calibrated combustible gas analyzer were similar to or slightly greater than the nominal values.

Reliability: (1) Reliable without restrictions

Flag: Key study for SIDS endpoint.

References: Rinehart, W.E. (1967) Toxicological Studies on Several Alpha Olefins. University of Pittsburgh, submitted to Gulf Research and Development Co. (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(2) Test Substance

Identity (purity): CAS No. 68526-53-4; Alkenes, C6-8, C7 rich

Method

Method/guideline: Not specified
Type *(test type):* LC50
GLP: Pre-GLP
Year: 1979
Species/Strain: Swiss albino Mice, Sprague-Dawley Rats, Hartley Guinea Pigs
Sex: Males and Females
No. of animals per sex per dose: 5

Vehicle: None
Route of administration: Inhalation

Test Conditions: Age of the test animals was not reported. Body weights ranged from 17 to 23g (mice), 187 to 260g (rats), and 293 to 381g (guinea pigs) at initiation of the study. Animals were given single doses of test material vapor at a concentration of 42.3 mg/L for 6 h. Control animals (5/sex/species) were exposed to clean air as a sham exposure.

Room air, at a flow rate of 134 L/minute was bubbled through test material in a flask to produce a vapor-laden airstream that was directed, undiluted, into the exposure chamber. The nominal exposure concentration was calculated by dividing the mass of test material consumed by the total volume of air passing through the chamber. For the purpose of this study, the test material was considered to be free of impurities.

Animals were observed throughout the exposure period for signs of toxicity. Following the exposure period, animals were observed for signs of toxicity daily for 14 days. Body weights were recorded on Days 0, 1, 2, 4, 7, and 14. Gross necropsies were performed on any animals that died during the study and all animals at the completion of the study. During the necropsies, the lungs with trachea, kidneys, and liver were preserved for possible histopathological examination.

The statistics used to analyze the data were not reported.

Results:

Value: LC50 > 42.3 mg/L for 6 h (10,533 ppm)

Number of deaths at each dose level: One female mouse died 1 hr into the exposure period. Two guinea pigs (1 male and 1 female) died by 45 minutes into the exposure period.

Remarks: In mice, exposure to 42.3 mg/L of the test substance resulted in one female death one hour into the exposure period. All other mice survived until the end of the study. None of the rats died during the study. Two guinea pigs (one male, one female) died by 45 minutes into the exposure period. The remaining guinea pigs survived until the end of the study. All exposed species exhibited signs of systemic toxicity including labored breathing, prostration, body tremors, and ataxia during the exposure. However, in the surviving animals, these signs completely reversed within 24 hours following the exposure. Liver discoloration was noted upon necropsy in the mouse and the two guinea pigs that died during the exposure. Otherwise, no significant findings were observed at necropsy. Under conditions of this study, Alkenes, C6-8, C7 rich have a low order of acute inhalation toxicity in rodents.

Reliability: (1) Reliable without restrictions

Flag: Key study for SIDS endpoint

References: Bio/dynamics, Inc. (1979) An Acute Inhalation Toxicity Study of MRD-ECH-78-32 in the Mouse, Rat, and Guinea Pig. Conducted for Exxon Research and Engineering Company (unpublished report).

(3) **Test Substance**

Identity (purity): CAS# 558-37-2, Neohexene (3,3-dimethylbutene-1, 98.5% purity).

Method

Method/guideline: OECD 403
Type *(test type):* LC50
GLP: Not specified
Year: 1982
Species/Strain: Rat/Sprague-Dawley
Sex: Males and Females
No. of animals per sex per dose: 5

Vehicle: None
Route of administration: Inhalation

Test Conditions: One group of five rats/sex (mean body weight: 276.6 g for male, 206.6 g for female) was placed in a 38 liter exposure chamber and exposed for four hours to the maximum practical vapor concentration (51,000 ppm). Analytical chamber concentrations were measured using a total hydrocarbon monitor (method or frequency not specified). The animals were observed hourly during the exposure and twice daily for a period of 14 days for mortality and signs of systemic toxicity. Body weights were recorded prior to treatment and at 2, 3, 4, 7, and 14 days. The animals were necropsied at the end of the 14-day period and observed for gross abnormalities. Statistical methods were not specified

Results:

Value: LC50: >51,000 ppm (176 mg/L)
Number of deaths at each dose level: No animals died during the study.

Remarks: The mean analytical exposure concentration was 51,000 ppm. All the rats were observed prostrate in their cages during the exposure. All animals appeared normal throughout the post-exposure observation period. All animals gained weight during the study except the females at the Day 3 interval (slight group mean weight loss). There were no significant findings at necropsy.

Reliability: (1) Reliable without restrictions

References:	Hazleton Laboratories America, Inc. (1982) Neohexene: Acute Inhalation Toxicity Test in Rats. Conducted for Phillips Petroleum Company (unpublished report).

C. Acute dermal toxicity

(1) Test Substance

Identity (purity):	CAS No. 592-41-6, 1-Hexene (NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 402 [except that four males and four females were listed]
Type *(test type):*	LD50
GLP:	Yes
Year*:*	1982
Species/Strain:	Albino rabbits/New Zealand White
Sex:	Males and females
No. of animals per sex per dose:	4
Vehicle:	None
Route of administration:	Dermal
Test Conditions*:*	No data

Results:

Value:	LD50 > 2 g/kg
Number of deaths at each dose level:	No mortalities were observed.
Remarks:	No deaths or signs of systemic toxicity at 2.0 g/kg; no treatment-related gross pathology except at application site. Skin irritant effects observed in the test animals included minimal erythema and edema following removal of the wrappings at 24 hours. No skin irritant effects were evident at 14 days and body weight gain was not affected.
Reliability:	(1) Reliable without restrictions
Flag:	Key study for SIDS endpoint
References:	Shell Development Company (1982) Acute Dermal Toxicity of NEODENE 6 Alpha Olefin in the Rabbit, WTP-124 (unpublished report).
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(2) **Test Substance**

Identity (purity): CAS No. 68526-53-4; Alkenes, C6-8, C7 rich

Method

Method/guideline: Not specified
Type *(test type):* LD50
GLP: Pre-GLP
Year: 1978
Species/Strain: New Zealand White rabbits
Sex: Males and females
No. of animals per sex per dose: 2

Vehicle: None
Route of administration: Dermal

Test Conditions: Test animals were at least 9 weeks old and weighed between 2.2 and 3.3kg at the start of the study. Concentration levels were 200 and 3160 mg/kg. Undiluted test material was applied to clipped, abraded abdominal skin under gauze and thick plastic. Following the 24-hour exposure period, the wrapping was removed and the exposed area was wiped to remove residue. Animals were observed for gross signs of irritation and systemic toxicity 1,2,3, and 4 hours post dose and daily for 7 days. Following the post-exposure observation period, animals were weighed, sacrificed and necropsied. Throughout the study, food and water were available at all times and animals were housed individually. Statistics used to evaluate the data were not reported.

Results:

Value: LD50 > 3160 mg/kg
Number of deaths at each dose level: No mortalities were observed at any dose tested.

Remarks: Lethargy and ataxia were observed in all animals, but these symptoms cleared by Day 2. Dermal reactions were generally moderate at 200 mg/kg and cleared by Day 14. In the high dose group, more severe dermal reactions, including moderate edema and severe erythema, persisted through the study. No significant fluctuations in body weight occurred. Necropsy findings were unremarkable except for a pus-filled liver in 1 rabbit from the high dose group. Under the conditions of this study, Alkenes, C6-8, C7 rich have a low order of acute dermal toxicity.

Reliability: (1) Reliable without restrictions

Flag:	Key study for SIDS endpoint
References:	MB Research Laboratories, Inc. (1978) Alkenes, C6-8, C7 Rich: Acute Dermal Toxicity in Albino Rabbits (unpublished report).

D. Acute toxicity, other routes

No data available

5.3 Corrosiveness/Irritation

A. Skin Irritation/Corrosion

(1) Test Substance: CAS No. 592-41-6, 1-Hexene (NEODENE 6 alpha olefins)

PH: Not applicable

Method

Test Type: OECD 404 except that the exposure was 24 hours, dressing was occlusive, and skin was evaluated only at 24 and 72 hours.

GLP: Yes
Year: 1982

Test Conditions

Species: Rabbits
Strain: New Zealand White
Cell type:
Sex: Males and females
Number of animals per sex per dose: 3

Total dose: 0.5 ml undiluted test substance per site
Vehicle: None
Exposure time period: 24 hrs
Grading scale: Draize

Method Remarks: Irritancy observed at 24 and 72 hours. Six New Zealand White rabbits, obtained from Nichols Rabbitry, Lumberton, TX, were clipped of all hair of the back/trunk area 24 hours prior to application of test substance. On dosing day (Day 0) the exposed region of the rabbbit's back was divided into four quadrants. Immediately prior to exposure, the areas within quadrants 2 and 3 were abraded using a 20 gauge hypodermic needle. Abrasion, made in a "tic-tac-toe" pattern were deep

enough to penetrate the stratum corneum, but not deep enough to penetrate the underlying derma or to cause bleeding. Areas of quadrants 1 and 4 were left intact. A 0.5 ml sample of undiluted test material was applied to each of four 1 by 1 inch squares of surgical gauze, and then placed on the skin of each exposure site. Patches were held in place with Blenderm tape. The entire trunk area was covered in impervious covering, secured with Blenderm, and wrapped with an elastic bandage. After 24 hours, all dressings were removed and the test area was wiped with a moist towel. Fifteen to twenty minutes after removal of dressings, the exposure sites were evaluated for erythema and edema using the Draize method. Sites were also evaluated at 72 hours following exposure using the same scoring table.

Results: The maximum score for any animal was at 24 hours and was 2 for erythema and 0 for edema. The Draize primary irritation score (range 0 - 8.0) was 0.975.

Reliability: (1) Reliable without restrictions

Reference: Shell Development Company (1982) Primary Skin Irritation of NEODENE 6 Alpha Olefin in the Rabbit, WTP-121 (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(2) **Test Substance:** CAS No. 592-41-6, 1-Hexene (Shop Olefin C6), >99% 1-hexene

PH: Not applicable

Method: 4-hr skin irritancy

Test Type: In-vivo
GLP: No
Year: 1985

Test Conditions

Species: Rabbits
Strain: New Zealand White
Cell type:
Sex: Male and female, aged 3-6 months
Number of animals per sex per dose: 3

Total dose: 0.5 ml undiluted test material
Vehicle: None
Exposure time period: 4 hr

	Grading scale:	Draize
	Method Remarks:	Irritancy observed at 24, 48 and 72 hours. Dorsal hair between the shoulders and hindquarters was shaved. A 2 cm x 2 cm lint patch with 0.5 ml of the test material was applied. The patch and surrounding skin were covered by a single layer of gauze and held in place with elastic adhesive bandage.
	Results:	4 hour skin irritancy observed at 24, 48 and 72 hours was 0 for erythema and edema.
	Reliability:	(1) Reliable without restrictions
	Reference:	Shell (1985) Toxicology of Shop Olefin: The Skin Irritancy of Shop Alpha Olefin C_6; C_{18}; and Shop Olefins 103, SBGR 85.166 (unpublished report).
	Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.
(3)	**Test Substance:**	SHOP C68 Internal Olefin
	Remarks:	CAS No. 25377-72-4, (Pentene=1.9%); CAS No. 25264-93-1, (Hexene=43.3%), CAS No. 25339-56-4, (Heptene=21.7%) and 25377-83-7 (Octene=31.7%), and CAS No. 27215-95-8 (Nonene=1.4%)
	Method:	OECD Guidelines, Section 404 and Section B4 of Directive 92/69/EEC
	Test Type:	in vivo
	GLP:	Yes
	Year:	1995
	Test Conditions	
	Species:	Rabbits
	Strain:	New Zealand White
	Cell type:	
	Sex:	Female
	Number of animals per dose:	3
	Total dose:	0.5 ml
	Vehicle:	None
	Exposure time period:	4 hrs
	Grading scale:	Draize
	Method Remarks:	At the start of the study, the three month old animals supplied by Froxfield SPF Rabbits, Hampshire, England, weighed 2.37 to 2.68 kg. Animals had free access to a commercially available

standard pelleted rabbit diet and tap water taken from the public supply. During the acclimatization period, the health status of each animal was monitored and a record kept. Each animal was examined for abnormality or irritation of the dermal test site before allocation to study. On the day before the dosing, the dorsum between the limb girdles was clipped (chemical depilatories were not used). Two test areas were marked on either side of the clipped area of dorsum. A single dose was applied directly to the skin and covered by an unmedicated gauze patch which was held in place on the left test site by strips of Blenderm. The right test site, acting as a control, was covered by a similar semi-occlusive dressing but otherwise remained untreated. Pads of cotton wool and elasticated bandage were used to protect the patches and ensure good contact between the skin and the test material during the four-hour exposure period. The elasticated bandage was held in place by thin strips of waterproof plaster (Blenderm) at both edges. Four hours after application, the treatment sites were gently washed with warm water and dried with paper tissues to remove excess test material adhering to the skin. Assessment of skin irritation responses at the control and treated test sites were made at 1, 24, 48, and 72 hours after removal of the bandages. Additional observations of persistent effects of treatment were made on Days 7, 10 and 13. Reactions of the test sites were assessed according to the criteria of Draize.

Results: The test material induced very slight to slight erythema and edema during the first 72 hours after bandage removal. Two animals had a score of 2 for erythema at 48 hours. There were no other dermal findings. Very slight erythema (score 1) persisted in one animal to Day 7. Exfoliation was evident in all animals on Day 7 and two on Day 10. The test sites of all animals were overtly normal on Day 13.

Reliability: (1) Reliable without restrictions

Reference: Huntington Life Sciences Ltd, (1996) SHOP C68 Internal Olefins: Skin Irritation in the Rabbit; Performed for Shell Chemical Co. (unpublished report).

B. Eye Irritation/Corrosion

(1) **Test Substance:** CAS No. 592-41-6, 1-Hexene (NEODENE 6 alpha olefin)

pH: Not applicable

Method: OECD 405, 3 male and 3 female – unwashed; 3 males - washed

Test Type: In-vivo
GLP: Yes

Year:	1982

Test Conditions

Species:	Albino rabbits
Strain:	New Zealand White
Cell type:	
Sex:	Male and female
Number of animals per dose:	9 (6 unwashed and 3 washed)
Dose(s) used:	One tenth milliliter of undiluted test material was placed into the right eyes of 3 male and 3 female rabbits. The eyes of an additional group of 3 male rabbits was flushed with tap water 30 seconds after exposure.
Vehicle:	None
Observation period:	The eyes were examined and scored for irritation one hour and 1, 2, 3, and 8 days after treatment.
Scoring method used:	Draize
Results:	The maximum total Draize score for any animal was 8 at 1 hour. The maximum average total Draize score (range 0 - 110) occurred at 1 hour, and was 5.0 for unwashed and 5.3 for washed eyes.
Remarks:	Washing did not reduce irritation.
Reliability:	(1) Reliable without restrictions
Reference:	Shell Development Company (1982) Eye Irritation of NEODENE 6 Alpha Olefin in the Rabbit, WTP-122 (unpublished report).
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(2) **Test Substance:** SHOP C68 Internal Olefin

Remarks:	CAS No. 25377-72-4, (Pentene=1.9%); CAS No. 25264-93-1, (Hexene=43.3%), CAS No. 25339-56-4, (Heptene=21.7%) and 25377-83-7 (Octene=31.7%), and CAS No. 27215-95-8 (Nonene=1.4%)
Method:	OECD Section 405 and Section B5 of Directive 92/69/EEC
Test Type:	in vivo
GLP:	Yes
Year:	1995

Test Conditions

Species:	Rabbits
Strain:	New Zealand White
Cell type:	
Sex:	Female
Number of animals per dose:	3
Dose(s) used:	0.1 ml
Vehicle:	None
Observation period:	72 hrs
Scoring method used:	Draize scoring at 24, 48, and 72 hours after treatment
Remarks:	At the start of the study, the four month old animals supplied by Froxfield SPF Rabbits, Hampshire, England, weighed 2.80 to 3.35 kg. Animals had free access to a commercially available standard pelleted rabbit diet and tap water taken from the public supply. During the acclimatization period, the health status of each animal was monitored and a record kept. Each animal was subjected to a single ocular instillation of 0.1 ml of the test material. Ocular reactions were assessed 1, 24, 48, and 72 h ours after treatment.
Results:	Very slight or slight conjunctivitis was observed in all animals one hour after instillation, persisting in one animal to the 48 hour examination. The treated eye of each animal was overtly normal by the 72 hour examination. At hour 1, one animal had a score of 2 for redness, two had scores of 1. At 24 hours, 1 rabbit had scores of 1 for redness, 2 animals had scores of 0. At 48 hours, 1 animal had scores of 1 for redness, all other scores were 0. At 72 hours, all scores were 0.
Reliability:	(1) Reliable without restrictions
Reference:	Huntington Life Sciences Ltd, (1996) SHOP C68 Internal Olefins: Eye Irritation in the Rabbit; Performed for Shell Chemical Co. (unpublished report).

5.4 Skin Sensitisation

A. **Test Substance:** CAS No. 592-41-6, 1-Hexene (1% w/w in ethanol, NEODENE 6 alpha olefin)

Method:	OECD 406 - Buehler
Test Type:	In-vivo
GLP:	Yes
Year:	1982

Test Conditions

Species:	Guinea pig
Strain:	Duncan-Hartley albino
Sex:	Male and female
Number of animals per sex per dose:	5
Route of administration:	Topical occlusive
Induction conc.:	1%
Induction vehicle:	ethanol
Challenge conc.:	1%
Challenge vehicle:	ethanol
Grading system used:	0 = no reaction +/- = minimal erythema 1 = slight erythema 2 = moderate erythema 3 = moderate erythema with slight edema 4 = severe erythema with moderate edema
Method remarks:	DNCB was used as a positive control. A preliminary test was conducted during the third week of the pre-trial period using 6 animals at three different concentrations (1 male, 1 female at each concentration) to determine the non irritating dose level. Groups of 5 male and 5 female Duncan Hartlley albino guinea pigs, weighing 399 to 461 grams, were treated with 0.5 ml of 1% w/w 1-hexene in absolute ethanol, 0.5 ml of 0.1% w/v 2,4-dinitrochlorobenzene, or 0.5 ml absolute ethanol. The exposure sites were shaved (48 hours) and depilated (24 hours) prior to exposure. Sensitizing doses were applied topically under occlusive bandages (gauze pad secured with Blenderm surgical tape) one day a week, six hours per day for 3 consecutive weeks. After a two-week rest period following the last sensitizing dose, a challenge dose was given in the same manner as the sensitizing dose on the original site and on a virgin site. At this time only, a separate group was treated with 0.5 ml 1% w/w 1-hexene in absolute ethanol at one site to serve as an irritation control.
Results:	Negative for sensitization
Grades:	0 for all animals
Results Remarks:	Number of animals with skin reaction at challenge: 0/10 Number of animals with skin reaction in control group at challenge: 0/10 The control group exhibited slight irritation caused by ethanol; the scores decreased over the 5 week period from 0.03 at week 1 to 0.00 at week 5. The average skin reaction scores for the positive control group were 0.53 at week 1 and 1.34 at week 5, indicating a positive skin sensitizing reaction. The test group exhibited slight irritation caused by 1-hexene; however, the group had only one +/- score at week 1 and week 3. The scores decreased over the 5 week period from 0.03 at week 1 to 0.00 at week 5. No irritation was observed in the irritation control group.

	Reliability:	(2) Reliable with restrictions: Animals were examined for health and suitability five days prior to treatment rather than "within 3 days prior to test." Protocol indicated that a total of 48 guinea pigs would be required for the study; however, only 46 animals were used.
	Reference:	Shell Development Company (1982) Guinea Pig Skin Sensitization of NEODENE 6 Alpha Olefin, WTP-123 (unpublished report).
	Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.
B.	**Test Substance:**	SHOP C68 Internal Olefin
	Remarks:	CAS No. 25377-72-4, (Pentene=1.9%); CAS No. 25264-93-1, (Hexene=43.3%), CAS No. 25339-56-4, (Heptene=21.7%) and 25377-83-7 (Octene=31.7%), and CAS No. 27215-95-8 (Nonene=1.4%)
	Method:	OECD Guidelines, Section 406 and Section B6 of Directive 92/69/EEC
	Test Type:	Magnusson and Kligman
	GLP:	Yes
	Year:	1995
	Test Conditions	
	Species:	Albino Guinea pig
	Strain:	Dunkin-Hartley
	Sex:	Males and females
	Number of animals per sex:	10 each in test group; 5 each in control group
	Route of administration:	Topical
	Intradermal Induction conc.:	50%
	Intradermal Induction vehicle:	Paraffin oil
	Topical Induction conc.:	100%
	Topical Induction vehicle:	none
	Challenge conc.:	100% and 30%
	Challenge vehicle:	Paraffin oil
	Positive control:	none
	Grading system used:	Draize
	Method remarks:	The dorsal trunk and flanks of the 6-8 week old animals, weighing 302-380 grams were clipped on the day prior to dosing. Four males and four

females were dosed at 0.4 ml/site at concentrations of 2.5%, 5%, 10%, 25%, and 50% under occlusion with test material for a period of six hours and examined and graded in accordance with the Draize method at 24 and 48 hours after completion of exposure.

A Test Group of 10 male and 10 female animals was dosed topically at 0.4 ml/site under occlusion with test material once per week for three weeks, a total of three induction exposures. A Positive Control Group of 6 males and 6 females was dosed with dinitrochlorobenzene (DNCB). Doses were applied under 25-mm Hill Top Chambers®, with adhesive backs removed, occluded with plastic wrap and overwrapped with Elastoplast® tape. The period of exposure was six hours, after which the bandages were removed, and the sites wiped with disposable paper towels moistened with tepid tap water. The test material concentration used for induction and dosing (100% and 0.1% in acetone, respectively) were selected based on the irritation rangefinding phase.

Two weeks after the last induction exposure, Test Group animals were challenge dosed by topical application of a known essentially nonirritating concentration of the test material to previously unexposed areas of skin for six hours. The Positive Control Group was induced and challenged on a similar regimen as the Test Group with DNCB. Reactions to challenge dosing were evaluated at approximately 24 and 48 hours after completion of each exposure.

Results: Negative for sensitization

Grades: See remarks

Results Remarks: Intradermal injection of 50% v/v SHOP C68 in paraffin oil gave rise to isolated cases of slight or moderate erythema and pallor; a similar administration of 50% v/v SHOP C68 in the adjuvant resulted in slight or moderate erythema, pallor, discoloration, eschar formation and edema. The entire suprascapular region of five animals was edematous.

Occluded topical induction application of SHOP C68 gave rise to slight erythema and exfoliation.

Challenge application of test material gave rise to a positive response (slight erythema or a more marked reaction) in sixteen test and four control animals. Challenge of test material in paraffin oil caused a positive response in five test and no control animals. Challenge application of paraffin oil alone caused a positive response in one test and no control animals. Re-challenge application of 50% v/v SHOP C68 in paraffin oil caused a positive response in two test and one control animals. Re-challenge application of paraffin oil alone caused a positive response in one test and no control animals.

Although the incidence of significant responses was slightly higher in test than control animals, the difference was not considered to be sufficiently marked to be attributed to contact sensitization. The

reactions were considered to reflect primary irritation. It was therefore concluded that, under the conditions of this study, repeated administration of SHOP C68 did not cause delayed contact hypersensitivity in guinea pigs.

Reliability: (1) Reliable without restrictions

Reference: Huntingdon Life Sciences Ltd. (1996) SHOP Internal Olefin: Delayed hypersensitivity study in the Guinea Pig (Magnusson Kligman Method), Performed for Shell Chemical Co. (unpublished report).

5.5 Repeated Dose Toxicity

A. Test Substance[MM3]

Identity:	CAS No. 68526-52-3, Alkenes C6
Remarks:	Composition: C5 n-olefins = 0.5%, C5 iso-olefins = 1.3%, C6 n-olefins = 10.4%, C6 iso-olefins = 55.6%, C5 n-paraffins = 3.3%, C5 iso-paraffins = 9.3%, C6 iso-paraffins = 17.8%, C7 iso-olefins = 1.0%

Method

Method/guideline:	OECD 422
Test type:	Combined repeated dose toxicity study with reproduction/developmental toxicity screening test
GLP:	Yes
Year:	2002
Species:	Rat
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR
Route of Administration:	Oral gavage
Duration of test:	Up to 38 days (general systemic toxicity and neurotoxicity); see Remarks
Doses:	0, 100, 500, or 1000 mg/kg b.w./day
Sex:	Males and females
Exposure period:	Minumum of 28 days and up to 38 days, see Remarks
Frequency of treatment:	Once daily
Control group and treatment:	Concurrent vehicle control (corn oil)
Post exposure observation period:	None
Statistical methods:	Data for the toxicity study, including body weights, body weight gain, food consumption, were analyzed by One-Way Analysis of Variance (ANOVA). If significance was detected ($p<0.05$), pairwise group comparisons were performed using the Tukey-Kramer test. Descriptive (categorical) data and quanta data were analyzed by Fisher's Exact Test. When significance was observed, group by group comparisons were performed using Fisher's Exact Test. Absolute and relative organ weights, and clinical pathology data were analyzed for homogeneity of

variance using Levene's test. If significance was detected with Levene's test ($p<0.01$), multiple group comparisons proceeded using the Kruskal-Wallis non-parametric ANOVA, followed by Dunn's test, when $p<0.05$. If significance was not detected with Levene's test, parametric procedures were used to analyze the data, i.e., ANOVA followed by Tukey-Kramer test when $p<0.05$. All analyses were two-tailed with a minimum significance level of 5% ($p<0.05$).

Test Conditions:

This study was conducted to: (1) provide screening information on the repeated-dose systemic toxicity of the test substance, with emphasis on potential neurological effects, and (2) serve as a screening study for potential reproductive and developmental effects in male and female rats.

GENERAL SYSTEMIC TOXICITY PHASE (see Sections 5.9.A(1) and 5.9.B(1) for the reproductive toxicity phase): On the day following receipt, animals were approximately 9 wks of age and weighed 237 – 296 g (males) and 170 – 226 g (females). Animals were acclimated for 17 days prior to dosing. The study consisted of one control group and three treatment groups with 12 animals in each group. Animals were treated for a minimum of 4 wks, up to and including the day prior to scheduled euthanasia. Detailed clinical observations were performed a minimum of weekly and on the day of scheduled euthanasia. An abbreviated functional observation battery (FOB) was performed prior to study initiation and weekly thereafter. A full FOB was performed following 28 days of treatment. Individual body weights were recorded on days 0, 3, 7, 12, 16, 20, 23, 27 and 30. A final body weight was recorded prior to scheduled euthanasia (day 34/38) Individual food consumption was recorded on the same days as body weights (except for males during cohabitation with females in the reproduction/ developmental screening study). Blood samples were collected on the day of scheduled euthanasia (day 34/38) for evaluation of selected hematology, coagulation and clinical chemistry parameters. All animals were subjected to a complete gross necropsy examination at the time of death or scheduled euthanasia (day 34/38). Organ weights (liver, kidneys, testes, epididymides, adrenals, thymus, spleen, brain and heart) were recorded from surviving animals and the following tissues and organs were preserved from all animals: all gross lesions, accessory genital organs, adrenals, aorta, brain, cecum, colon, duodenum, esophagus, exorbital lachrymal glands, eyes with optic nerve, femur, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, mesenteric/mandibular/ mediastinal lymph nodes, ovaries, pancreas, peripheral nerve, pituitary, rectum, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, submaxillary salivary gland, testes, thymus, thyroid, parathyroid, tongue, trachea, and urinary bladder. All tissues and organs collected at necropsy from animals in the control and high-dose groups and animals found dead, and gross lesions from animals in the low- and mid-dose groups were examined microscopically.

Results

NOAEL (NOEL):

NOAEL (general systemic toxicity) = 1000 mg/kg/day (study author assigned)
NOEL (general systemic toxicity) = 100 mg/kg/day for females due to kidney effects; none for males due to kidney and adrenal effects (reviewer assigned)
LOAEL (general systemic toxicity) = 500 mg/kg/day for females and 100 mg/kg/day for males (reviewer assigned)
NOEL (neurotoxicity) = 1000 mg/kg/day (reviewer assigned)

Actual dose received by dose level by sex if known:

As administered. Analysis of dosing mixtures confirmed that mixtures were accurately prepared.

Remarks:

No test article-related mortality occurred. Post-dose salivation was observed for males (11/12) and females (6/12) in the 1000 mg/kg/day group. Remarkable clinical signs in the 500 mg/kg/day group were limited to a single incidence of post-dose salivation in 1 male. No remarkable clinical signs were observed for females in the 500 mg/kg/day group or males or females in the 100 mg/kg/day group. No toxicologically meaningful differences were noted in the FOB evaluations.

There were no toxicologically meaningful differences noted in mean body weights, body weight gain, food consumption, hematology, or coagulation or the clinical chemistry parameters evaluated. All significantly different values were within the laboratory's range of historical control data values. The mean prothrombin time of females in the 100 mg/kg/day group was statistically lower than controls on day 38. This difference did not follow a consistent pattern and was not dose responsive. Statistically significant higher mean total protein, potassium, calcium, phosphorus and albumin values were noted for males in the 1000 mg/kg/day group on day 34. In females, statistically significant differences in clinical chemistry data included a lower mean AST value in the 500 mg/kg/day group and a higher mean phosphorus value in the 1000 mg/kg/day on day 38.

No remarkable gross necropsy findings were noted in the control or test article-treated groups. A few statistically significant differences in organ weights were noted; however, none of the differences were considered toxicologically meaningful since they did not correlate with any toxicologically significant histopathological changes. In males, statistically significant differences in organ weight data included higher absolute adrenal weight in the 100 mg/kg/day group; higher absolute kidney weights in the 100, 500 and 1000 mg/kg/day groups; higher kidney weight relative to final body weight in the 500 and 1000 mg/kg/day groups; and higher liver weight relative to final body weight in the 1000 mg/kg/day group. In females, statistically significant differences in organ weight data included higher kidney weight relative to final body weight in the 500 and 1000 mg/kg/day groups, and higher liver weight relative to final body weight in the 1000 mg/kg/day group.

Minimal to mild hyaline droplet nephropathy within the proximal convoluted tubules was observed in 9 of 12 males in the 1000 mg/kg/day group. Accumulation of α2μ –globulin protein within the proximal convoluted tubule epithelial cells can be induced by a variety of chemical compounds that reversibly bind with α2μ-globulin protein, thus decreasing the rate of protein complex metabolism by the cells. Tubular epithelial degenerative and regenerative changes commonly accompany the accumulation of hyaline droplets; however, its presence and associated nephropathy in male rats is not considered toxicologically significant for humans.

(See Sections 5.9 A(1) and 5.9.B(1) for Reproductive and Developmental results and Section 5.10.B.(1) for neurotoxicity results.)

Reliability: (1) Reliable without restrictions.

Flag: Key study for SIDS endpoint.

References: Thorsrud, B.A. (2003) A combined repeated dose toxicity study and reproduction/developmental screening study in Sprague Dawley rats with (C6) alkenes, Study No. 3604.2, Springborn Laboratories, Inc., Ohio Research Center, Spencerville, Ohio; conducted for American Chemistry Council (Higher Olefins Panel) (unpublished report).

B. Test Substance

Identity (purity): CAS No. 592-41-6, 1-Hexene (90 – 100%, NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 413
Test type:	90-day subchronic inhalation toxicity study
GLP:	Yes
Year:	1984
Species:	Rat
Strain:	F344
Route of Admin.:	Inhalation – vapor
Duration of test:	90 days
Doses:	0, 300, 1000, 3000 ppm (0, 1033, 3442 and 10,326 mg/m^3)
Sex:	Males and females
Exposure period:	6 hr/day
Freq. of treatment:	5 days/week, 13 weeks
Control group:	Air exposed
Post exposure observation period:	Not applicable
Statistical methods:	Unadjusted body weights were analyzed by Dunnet's test. Organ weights, clinical chemistry, hematology, urinalysis, and organ-to-body weight ratio data were analyzed by Dunnett's t-test on ranked data. The Rotorod data for neuromuscular coordination were adjusted by summing the time for the best 3 of 4 trials for each rat.

Test Conditions: The objective of this study was to evaluate the toxicity of 1-hexene following repeated inhalation exposures in male and female Fischer 344 rats. Groups of 40 male and 40 female rats (young, 125-160 g at study initiation), were exposed for 6 hours per day, 5 days per week, over a 13-week period. Treatment groups (10 rats/sex/group) consisted of air-exposed control (0 ppm) and three test groups of 300, 1000, and 3000 ppm 1-hexene. During the treatment period, the rats were observed daily for clinical signs of toxicity; body weights and neuromuscular coordination [females only] were measured at 7-day intervals. After 7 weeks of exposure and at the end of the treatment period, the rats were examined for macroscopic pathology (lungs, liver, kidneys, brain, heart, spleen, right testicle without epididymides), for microscopic pathology (brain, right median nerve, right sciatic nerve, nasal turbinates, trachea/larynx, lungs, liver, kidneys, right testicle, and epididymides), clinical chemistry, hematology, urinalysis, and sperm counts.

Results

NOAEL (NOEL): NOEL = 1000 ppm (3.442 mg/L), based on changes in bodyweight (females) and questionable organ weight changes in both sexes at 3000 ppm

LOAEL (LOEL): LOEL = 3000 ppm (10.326 mg/L)

Actual dose received by dose level by sex (if known): 0, 300, 1000, 3000 ppm

Remarks: No mortalities were observed during the course of the study. No clinical signs of toxicity attributable to 1-hexene exposure were observed. Female rats exposed to 3000 ppm had significantly lower body weights compared to control rats from exposure day 5 persisting throughout the treatment period.

Several statistically significant effects in hematology, clinical chemistry, and urinalysis evaluations were observed: elevated serum phosphorus in males at 300, 1000 and 3000 ppm and in females at 1000 and 3000 ppm; lower serum lactate dehydrogenase in female rats exposed to 1000 ppm, and in both male and female rats exposed to 3000 ppm; lower serum albumin in female rats exposed to 3000 ppm; elevated hematocrit and RBC count in 3000 ppm males and in 1000 and 3000 ppm females; lower mean corpuscular hemoglobin and hemoglobin concentration in 1000 and 3000 ppm females. These findings were either of small magnitude or did not correlate with histopathological findings, and thus did not appear to be of biological significance.

At 3000 ppm, male rats exhibited slightly increased absolute and relative testicular weights; however, when the left testicle was detunicated prior

to weighing, there was no statistically significant increase in testis weight compared with the controls. Female rats had slightly decreased absolute (but not relative) liver and kidney weights, at 3000 ppm. No treatment-related gross or histological lesions were noted in these or other tissues at either the interim or terminal sacrifice. Sperm counts were observed and were not considered to show statistical significance. (Please see Reproductive Toxicity section 5.9.A for further details about reproductive endpoints)

Exposure to 1-hexene did not affect neuromuscular coordination in females as determined using the Rotorod.

Reliability: (1) Reliable without restrictions

Flag: Key study for SIDS endpoint

References: Gingell, R., Bennick, J.E., and Malley, L.A. (1999) Subchronic inhalation study of 1-hexene in Fischer 344 rats. Drug Chem Toxicol. 22(3):507-28.

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

C. Test Substance

Identity (purity): CAS No. 592-41-6, 1-Hexene (90 – 100%, NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 407
Test type:	28-day oral repeated dose study
GLP:	Yes
Year:	1994
Species:	Rat
Strain:	Wistar
Route of Admin.:	Oral gavage
Duration of test:	28 days
Doses:	0, 10, 101, 1010, 3365 mg/kg/day
Sex:	Males and females
Exposure period:	28 days
Freq. of treatment:	daily for 28 days
Control group:	Dosed with water
Post exposure observation period:	none
Statistical methods:	No data

Test Conditions: Groups of 5 male and 5 female rats were dosed daily for 28 days with undiluted 1-hexene; controls were dosed with water.

Results

NOAEL (NOEL):	NOEL = 101 mg/kg/day for males (male rat-specific kidney effect) and 1010 mg/kg/day for females (gastric effects and spleen weights)
LOAEL (LOEL):	LOEL = 1010 mg/kg/day for males and 3365 mg/kg/day for females
Remarks:	The main effect of dosing was irritation of the gastric mucosa, as observed by macro- and microscopic examination at the top two dose levels (males and females). Body weights were reduced in males at these doses. Clinical signs of hunched posture and ruffled fur were seen at the top dose, probably reflecting general discomfort. Spleen weights were reduced at the top dose, but there were no associated histological findings. Opthalmoscopy, clinical chemistry, hematology, and neuromuscular coordination [by rotorod] were unaffected. Pathological changes were restricted to gastric effects.
Reliability:	(1) Reliable without restrictions
References:	Dotti, A., Duback-Powell, J.R., Biderman, K., and Weber, K. (1994) 4-week oral toxicity (gavage) study with 1-hexene in the rat. RCC Project 332695. Cited in HEDSET.
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

D. Test Substance

Identity (purity):	CAS No. 592-41-6, 1-Hexene
Remarks:	Three test articles were blended to produce the final test article consisting of 90-100% 1-hexene (NEODENE 6, GULFTENE 6 and alpha olefin 6)

Method

Method/guideline:	OECD 421 (modified) (see Section 5.9.A.2 for reproductive endpoints and Section 5.9.B.2 for developmental endpoints)
Test type:	Reproduction/Developmental Toxicity Screening Study
GLP:	Yes
Year:	1995
Species:	Rat
Strain:	Sprague-Dawley
Route of Admin.:	Oral gavage
Duration of test:	Males: 44 days; females: 41-55 days
Doses:	0, 10, 500, 1000 mg/kg/day
Sex:	Males and females
Exposure period:	28 days
Freq. of treatment:	daily
Control group:	Corn oil by oral gavage
Post exposure observation period:	none

Statistical methods: Continuous data, including body weights, body weight gain, feed consumption, organ weights, were analyzed by using a One-Way analysis of Variance. If significance [$P<0.05$] was detected, group by group comparisons were performed using Dunnett's test. All analyses utilized two-tailed tests for a minimum significance level of 5% comparing the control to the treated groups.

Test Conditions: 12 male rats (195-242 g, 6 weeks old) per group were exposed for 28 days prior to mating, and through mating until euthanasia for a total of 44 consecutive days of dosing; 12 females (163-219 g, 8 weeks old) per group were dosed for 14 days prior to mating, during mating, gestation and lactation through euthanasia at lactation day 4 [41-55 consecutive days]. Dose levels were 0, 100, 500, 1000 mg/kg/day in a corn oil vehicle [5 mL/kg]. Animals were observed daily for clinical signs of toxicity. Body weights and food consumption were determined weekly. Females that delivered were necropsied on lactation day 4. Females that failed to deliver were necropsied 25 days after evidence of mating was detected. For females, the ovaries and brain were weighed. For males, after 43 days of dosing, the viscera were examined, and brain, testes and epididymides weighed. The ovaries, testes, epididymides, liver, kidneys, and peripheral (sciatic) nerve of control and high dose animals, the kidneys of the low and mid dose animals, and all gross lesions from each group, were processed for microscopic examination.

Results

NOAEL (NOEL): NOEL for general toxicity in the P generation is <100 mg/kg/day for males (male kidney histopathology) and 1000 mg/kg/day for females

LOAEL (LOEL): LOEL = 100 mg/kg/day for males

Remarks: Please see Sections 5.9.A.2 and 5.9.B.2 for reproductive and developmental toxicity endpoints.

No mortality or clinical signs of toxicity were observed. For the F0 males and females at the top dose, gross and histological examination of the ovaries , testes, epididymides, liver, kidneys , and peripheral [sciatic] nerve was performed; kidneys were also examined at the mid and low dose levels. The only gross finding was pitted kidneys in a few mid and top dose males (2/12 in 500 mg/kg/day group and 3/12 in the 1000 mg/kg/day group, and the only histological finding was dose-related accumulations of hyaline droplets in the epithelial cells of the convoluted tubules of the kidneys of males (incidence of 0/0, 7/12, 8/12 and 9/12 for the 0, 100, 500 and 1000 mg/kg/day groups); no such effect was observed in female rats. Although there was no immunochemical verification, the author's concluded that the formed droplets were $alpha_{2u}$-globulin This condition was diagnosed as hydrocarbon nephropathy, which is considered specific to young adult male rats; there

is no indication that similar nephropathy will occur in humans exposed to 1-hexene.

Reliability: (1) Reliable without restrictions

References: Gingell, R., Daniel, E.M., Machado, M. and Bevan, C. (2000) Reproduction/developmental toxicity screening test in rats with orally-administered 1-hexene. Drug and Chem. Toxicology 23(2)327-338.

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

5.6 Genetic Toxicity *in vitro*

A. Gene Mutation

(1) Test Substance

Identity (*purity*): CAS No. 68526-52-3, Alkenes, C6

Method

Method/guideline:	EPA OTS 798.5265
Type:	in-vitro bacterial reverse mutation – Ames Assay
System of testing:	bacterial
GLP:	Yes
Year:	1991
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538
Metabolic activation:	With and without S9 fraction of livers from rats pretreated with Aroclor 1254
Concentrations tested:	3.2, 10, 32, 100 and 320 µg/plate (Doses were based on a pre-test for toxicity)
Statistical Methods:	The mean plate count and standard deviation for each dose point were determined. Any test value that was equal to or greater than three times the mean value of the concurrent vehicle control was considered to be a positive dose.

Test Conditions: For the purpose of this study, the test material was considered to be free of impurities. DMSO was the vehicle for controls. Ethanol was the vehicle for the test material. Vehicle controls were dosed at 0.1 ml/plate ethanol and 0.1 ml/plate DMSO. The positive controls were 2-Aminoanthracene, 9-Aminoacridine, 2-Nitrofluorene, N-methyl-N-nitro-N-nitrosoguanidine.

To determine the highest dose of compound to be used in the assay, a dose range from 1 to 10,000 µg/plate was tested. Only

strain TA98 was used. The toxicity pretest was repeated and toxicity was observed as a reduction in both background and revertant colony counts. 320 µg/plate was selected as the high dose to be used on the mutagenesis assay for both the saline (-S9) and the +S9 treated plates.

Triplicate plates were used for each dose level. A repeat assay was performed in order to verify the data produced in the initial assay.

Results

Cytotoxic conc.: 320 ug/plate

Genotoxic effects: Negative with and without metabolic activation

Remarks: The test material did not induce a dose related increase in the mutation frequencies of any of the tester strains either in the presence or absence of metabolic activation. All positive and negative controls responded in a manner consistent with data from previous assays. Under the conditions of this study the test material is not mutagenic for the Salmonella tester strains at doses up to and including 320 µg/plate.

Reliability: (1) Reliable without restrictions

Flag: Key study for SIDS endpoint.

References: Exxon Biomedical Sciences, Inc. (1991a). Alkenes, C6: Microbial Mutagenesis in *Salmonella*: Mammalian Microsome Plate Incorporation Assay. Conducted by Exxon Biomedical Sciences, Inc., East Millstone, NJ, USA (unpublished report).

(2) **Test Substance**

Identity (*purity*): CAS No. 592-41-6, 1-Hexene (99.06%)

Method

Method/guideline:	OECD 471 with repeat assay
Type:	In vitro bacterial reverse mutation – Ames Assay
System of testing:	bacterial
GLP:	Yes
Year:	1990
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538
Metabolic activation:	With and without 0.5 ml of S9 fraction of livers from Sprague Dawley rats pretreated with Aroclor 1254

Concentrations tested: 0.0015, 0.005, 0.015, 0.05, 0.15 and 0.5 mg/plate (Doses were based on a pre-test for toxicity)

Statistical Methods: Results were considered clearly positive if treatment with test material produced an increase in revertant colony numbers of at least twice the concurrent solvent control, with some evidence of a positive dose-relationship, in two separate experiments, with any bacterial strain either in the presence or absence of S9 mix. Results were considered clearly negative if treatment with test material did not produce reproducible increases of at least 1.5 times concurrent solvent controls, at any dose level with any bacterial strain.

When results fail to satisfy criteria for clear positive or negative response, repeat test may be performed using modifications. These modifications include the use of a narrower dose range and or the use of different levels of liver homogenate S9 fraction. If no clear positive response can be obtained, the test data will be subjected to analysis to determine statistical significance using analysis of variance followed by student's t test.

Test Conditions: PRELIMINARY TOXICITY ASSAY – Four concentrations of test substance were assayed for toxicity (5000, 500, 50, 5 ug/plate). Dimethylsulphoxide was used as the solvent and negative control. 0.1 ml of a bacterial culture containing approximately 2 x10^9 cells/ml and 0.5 ml of S-9 mix or 0.5 ml buffer were added to glass bijou bottles. 0.1 ml of test solution was added followed by 2 ml histidine deficient agar. The mixture was shaken and overlayed onto previously prepared plates containing minimal agar. Plates were incubated for three days at 37°C. Toxicity of the test substance was detected by a substantial reduction in revertant colony counts or by the absence of a complete background bacterial lawn.

MUTAGENICITY ASSAY – 0.1 ml aliquots of bacterial suspension and 0.5 ml of sterile buffer or S-9 mix were added to each of one set of sterile bijou bottles. 0.1 ml of test compound was added to the cultures at six concentrations. The appropriate positive control was added (Without S-9: 9-aminoacridine 80 ug/plate TA1537; N-ethyl-N'-nitrosoguanidine 3 ug/plate TA100; N-ethyl-N'-nitrosoguanidine 5 ug/plate TA1535; 2-nitrofluorene 1 ug/plate TA98; 2-nitrofluorene 2 ug/plate TA1538. With S-9: 2-aminoanthracene 0.5 ug/plate TA1538 and TA98; 1 ug/plate TA100; 2 ug/plate TA1535 and TA1537). Three replicates were used at each dose level. 2 ml of histidine deficient agar was added to each of the bottles, mixed, and overlaid onto minimal agar. Plates were incubated for three days at 37°C. Colonies were counted using a Biotran Automatic Colony Counter, and mean number of revertant colonies per treatment group was assessed. A repeat assay was performed in order to verify the data produced in the initial assay.

Results

Cytotoxic conc.:	With metabolic activation: 5 mg/plate (all strains); 0.5 mg/plate (TA98) Without metabolic activation: 0.5 mg/plate
Genotoxic effects:	Negative with and without metabolic activation
Remarks:	The concentration of the test compound resulting in precipitation was not reported in summary.
Reliability:	(1) Reliable without restrictions
Flag:	Key study for SIDS endpoint
References:	Huntingdon Research Center (1990) 1-Hexene: Bacterial Mutation Assay. Sponsored by Ethyl (unpublished report).
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(3) **Test Substance**

Identity (*purity*):	CAS# 558-37-2, Neohexene (3,3-dimethylbutene-1, 98.5% purity).

Method

Method/guideline:	OECD 471 without repeat assay
Type:	In vitro bacterial reverse mutation – Ames Assay
System of testing:	bacterial
GLP:	Not specified
Year:	1982
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538
Metabolic activation:	With and without; S9 fraction (0.5 ml/plate) of livers from rats pretreated with Aroclor 1254 (500 mg/kg for 5 days)
Concentrations tested:	0, 32.3, 96.5, 289.5, 868.4, and 2605 µg/plate (doses were based on a pre-test for toxicity)
Statistical Methods:	A positive response was defined as a reproducible, dose-related increase in revertant colonies over three concentrations with the baseline increase twice the solvent control level.
Test Conditions:	Solvent control: dimethylsulfoxide (DMSO). Positive controls: N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG), 9-

aminoacridine (9-AA), 2-nitrofluorene (2-NF), 2-aminoanthracene (2-AA).

Five different *Salmonella* strains were tested in the presence and absence of rat liver S-9. The test substance was soluble in the solvent (dimethylsulfoxide, DMSO) at 100 mg/ml. Five dose levels were tested, with three plates per dose level. The maximum dose selected was 2605 µg/plate based on observed growth inhibition during an initial toxicity test. Concurrent positive controls were also tested with and without metabolic activation.

Results

Cytotoxic conc.: 2605 µg/plate in the initial toxicity test; cytotoxicity in the mutagenicity assay was not reported

Genotoxic effects: Negative with and without metabolic activation

Remarks: The concentration of the test compound resulting in precipitation was not reported. The test substance was not mutagenic in any of the five strains of *Salmonella* tested in the presence or absence of Aroclor-induced rat liver S9.

Reliability: (1) Reliable without restrictions

References: Hazleton Laboratories America, Inc. (1982). Neohexene: *Salmonella typhimurium* mammalian microsome plate incorporation assay. Conducted for Phillips Petroleum Company (unpublished report).

(4) Test Substance

Identity (*purity*): CAS No. 592-41-6, 1-Hexene (99.06%)

Method

Method/guideline: OECD 476
Type: In-vitro mammalian Cell Gene Mutation TK+/- Test
System of testing: non-bacterial
GLP: Yes
Year: 1990
Cell Line: Mouse Lymphoma L5178Y
Metabolic activation: With and without S9 fraction of livers from Sprague Dawley rats pretreated with Aroclor 1254

Concentrations tested: Preliminary toxicity - 10, 15, 30, 62.5, 125, 250, 500, 750, 1000 ug/ml
Mutation test – S-9 mix:
Test 1 -15, 30, 62.5, 125, 200, 300, 400, 500, 750 ug/ml

Test 2 – 15, 30, 62.5, 125, 200, 300, 350 ug/ml

Mutation test + S-9 mix:

Test 1 – 15, 30, 62.5, 125, 200, 300, 400, 500, 750 ug/ml

Test 2 – 10, 15, 30, 62.5, 125, 200, 300, 350 ug/ml

Statistical Methods: Analysis of variance of the mutant frequencies after the data had been log transformed. The difference between each treated group and the control mutant frequency was tested for significance by one-sided t-test. The criteria for a positive response included: the induction of at least two-fold increase in mutant frequency relative to the concurrent control; the demonstration of a statistically significant response; evidence of a dose-related response; and reproducible response.

Test Conditions: PRELIMINARY TOXICITY TEST - 3 ml aliquots of cell suspensions containing 1 x 10^6 cells/ml were dispensed into containers followed by 2ml of media or 2 ml of S-9 mix. 50 ul of compound solution or DMSO was then added. Two cultures per dose were prepared, one with S-9 and one without S-9. Cell suspensions were incubated at 37 °C for 3 hours. After incubation cells were washed with media and transferred to a bottle containing 30 ml growth media. Cell growth was monitored at 24 and 48 hours after treatment.

MUTATION TEST – The mutation test was carried out as described for the preliminary toxicity test with the following modifications. Two cell cultures were treated for each dose level. 12 ml of cell suspension and 8 ml of media or S-9 mix were dispensed into 50 ml centrifuge tubes. 200 ul of solvent control, test compound solution or positive control was added. After test incubation, cells were washed once and transferred to bottles containing 60 ml growth media. Cells were maintained in culture for 48 hours to allow for expression of induced mutation.
At least four treatment levels were chosen in which the cell survival was in the range of 100-10% relative to controls for subsequent cloning in agar and assessment of mutant colony numbers. Cultures outside this range were discarded. 600 cells were plated in cloning medium for estimation of viability and 3 x 10^6 cells in selective medium for quantitation of mutation. Plates were incubated at 37°C for 12 days. Colonies with a diameter greater than 200 um were counted.

The positive control compound for tests carried out in the absence of S-9 mix was ethyl methane sulphonate ata final concentration of 500 ug/ml. 20-methylcholanthrene was used as a positive control in the presence of S-9 mix at a final concentration of 2.5 ug/ml. The solvent in both cases was DMSO.

Results

Cytotoxic conc.: Cytotoxicity expressed as mean % of control growth in suspension

Concentration ug/ml	0	10	15	30	62.5	125	200	300	350	400	500	750	500/ 2.5*
-S-9													
Test 1	100	-	103	109	99	82	31	2	-	2	2	2	69
Test2	100	-	101	110	87	52	<1	<1	1	-	-	-	85
+S-9													
Test 1	100	-	89	88	41	71	74	16	-	3	1	1	40
Test 2	100	81	83	2	51	26	1	1	1	-	-	-	43

* cytotoxicity of positive control

Genotoxic effects: Negative with and without S-9

Back-transformed mean mutant frequency

Concentration ug/ml	0	10	15	30	62.5	125	200	300	Positive control
-S-9									
Test 1	89	-	-	102[a]	99[a]	80	104[a]	-	749[c]
Test 2	73	-	87	68	81	108	-	-	651[c]
+S-9									
Test 1	85	-	98	79	-	96	78	139[b]	593[c]
Test 2	88	98	86	-	96	82	-	-	377[c]

a – significance level compared to control, 5%
b – significance level compared to control, 1%
c – significance level compared to control, 0.1%

Remarks: Statistically significant increases in mutant frequency were observed in test 1 in the absence of S-9. However, these increases were small, less than 20% greater than controls, and were only found to be statistically significant due to the very low variability within negative control group. Only one statistically significant increase in mutant frequency was observed in test 1 at the highest concentration, in the presence of S-9. However, none of the criteria for a clear positive were fulfilled. Ethyl methane sulfonate and 20-methylcholanthrene, the positive controls, induced highly significant increases in mutant frequency in both tests. It is concluded that 1-hexene does not demonstrate mutagenic potential in this *in vitro* gene mutation assay.

Reliability: (1) Reliable without restrictions

Flag: Key study for SIDS endpoint

References: Huntingdon Research Center (1990) 1-Hexene in Vitro Mammalian Cell Gene Mutation Assay (TK+/-). Sponsored by Ethyl (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

B. Chromosomal Aberration

(1) Test Substance

Identity (*purity*): CAS No. 592-41-6, 1-Hexene (NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 473
Type:	In-vitro mammalian chromosome aberration test
System of testing:	non- bacterial
GLP:	Yes
Year:	1983
Cell line:	Chinese Hamster Ovary (CHO) cells
Metabolic activation:	With and without S9 fraction
Concentrations tested:	No data
Statistical Methods:	No data

Test Conditions: Ethanol was the vehicle for the test material. Single cultures were used for the assay and cells were evaluated at 12 hours.
A repeat assay was performed in order to verify the data produced in the initial assay.

Results

Cytotoxic conc.: With metabolic activation: 0.61 mg/ml
Without metabolic activation: 0.067 mg/ml

Genotoxic effects: Negative with and without metabolic activation.

Remarks: A single increase in aberrations was noted in the first assay (with S-9) but was not dose-related or reproduced in the second assay. There was no evidence that the cell cycle was not delayed.

Reliability: (2) Reliable with restrictions: Study was conducted at a reliable laboratory, but only limited data were available for evaluation.

Flag: Key study for SIDS endpoint

References: Shell Development Company (1983) In Vitro Chromosome Aberration Assay in Chinese Hamster Cells of NEODENE 6 Alpha Olefin, WTP-126 (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11.

(2) Test Substance

Identity (*purity*):	CAS No. 592-41-6, 1-Hexene (99.06%)

Method

Method/guideline:	OECD 473
Type:	In-vitro mammalian chromosome aberration test
System of testing:	non- bacterial
GLP:	Yes
Year:	1990
Cell line:	Cultured human lymphocytes
Metabolic activation:	With and without S9 fraction of livers from Sprague Dawley rats pretreated with Aroclor 1254
Concentrations tested:	15.6, 62.5 and 125 mg/mL
Statistical Methods:	The number of aberrant metaphase figures in each group was compared with the solvent control value using Fishers test. Any apparent dose related trend was analysed using Mantel's test.

Test Conditions: A preliminary solubility test showed that 1-hexene was miscible in dimethylsulphoxide (DMSO) at a maximum concentration of 100 mg/ml. A final concentration of 1000 ug/ml in culture medium was initially immiscible but became completely miscible upon incubation at 37° C for five minutes. Therefore, 1000 ug/ml was selected as the maximum achievable concentration for further analysis. Ethyl methane sulfonate (EMS) and cyclophosphamide (CP) were used as positive controls in the absence and presence of metabolic activation, respectively. Lymphocytes were separated from human blood, washed and suspended at a concentration of 1 x 10^6 cells per ml. 5 ml aliquots of the cell suspension were incubated at 37°C in a humid atmosphere containing 5% CO_2 for approximately 48 hours. After 48 hours, 50 ul aliquots of 1-hexene were added to each of two duplicates to give final concentrations of 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9 and 2.0 ug/ml. The solvent control, DMSO, was added to 4 cultures in 50 ul aliquots and EMS, the positive control, was added to two cultures at a final concentration of 750 ug/ml. For testing in the presence of metabolic activation 1.25 ml of S-9 mix was added to each culture followed by 62.5 ul aliquots of various dilutions of 1-hexene giving the same final concentrations as above. DMSO (62.5 ul) was added to four cultures and CP was added to 2 cultures at a final concentration of 20 ug/ml. Cells were incubated for three hours after dosing. Cells were then centrifuged and resuspended in fresh medium and incubated for an additional 22 hours. Mitotic activity was arrested by addition of colchicines, cells were fixed and slides were prepared. Slides were examined by light microscopy and the proportion of metaphase figures in each culture was measured. The dose level causing a decrease of 50 –80% of the solvent control value or, if

no decrease, the maximum achievable concentration was used as the highest dose level for metaphase analysis. The intermediate and low dose were 50% and 12.5% of the highest concentration. Approximately 100 metaphase figures were examined from each culture.

Results

Cytotoxic conc.: Mitotic Index ([total # of mitotic cells/total # cells] x 100)

Concentration ug/ml	0	2	3.9	7.8	15.6	31.3	62.5	125	250	500	1000
-S-9	8	7.4	5.6	7.2	6.3	9.2	7.5	3.7	1.3	-*	-*
+S-9	11.3	9.3	9.7	9.0	8.5	8.4	7.4	6.6	1.5	-*	-*

*No live cells observed due to excessive toxicity

125 ug/ml reduced the mitotic index to 46% and 58% in the absence and presence of metabolic activity, respectively. Therefore, 125 ug/ml was selected as the high dose, while 62.5 and 15.6 ug/ml were selected as the intermediate and low doses.

Genotoxic effects: Negative with and without metabolic activation.

Test Material	Concentration ug/ml	No. cells examined	No. of aberrant cells (% mean)
-S-9			
DMSO	10 ul/ml	100	0.25
1-hexene	15.6	100	0.50
1-hexene	62.5	100	0.50
1-hexene	125	100	0.50
EMS	750	100	6.50*
+S-9			
1-hexene	15.6	100	0.00
1-hexene	62.5	100	0.50
1-hexene	125	100	0.50
CP	20	100	8.50*

* $P<0.001$

In both the presence and absence of metabolic activation, at all the concentrations of 1-hexene analyzed, no statistically significant increases in the proportion of metaphase figures containing chromosomal aberrations were observed. Both positive control compounds (EMS and CP) caused statistically significant increases in the proportion of aberrant cells, thus demonstrating the sensitivity of the test system and the efficacy of the S-9 mix.

Remarks: It is concluded that 1-hexene has shown no evidence of clastogenic activity in this *in vitro* cytogenetic test system.

Reliability: (1) Reliable without restrictions

References: Huntingdon Research Center (1990) 1-Hexene: Metaphase Chromosome Analysis of Human Lymphocytes Cultured in Vitro. Sponsored by Ethyl (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

C. Other Genetic Effects

(1) Test Substance

Identity (*purity*): CAS No. 592-41-6, 1-Hexene (GULFTENE 6)

Method

Method/guideline:	OECD 482 except that independent repeat was not conducted
Type:	In-vitro unscheduled DNA synthesis
System of testing:	Non-bacterial
GLP:	Yes
Year:	1984
Cell line:	Primary rat hepatocytes
Metabolic activation:	None

Concentrations tested: Rangefinding experiment: 32, 64, 128, 256, 512, 1024, 2048 and 5000 ug/ml
UDS experiment: 500, 2000, 3500 and 5000 ug/ml

Statistical Methods: The test substance was considered positive for unscheduled DNA synthesis (UDS) when the mean net nuclear grain count at any treatment level exceeded that of the concurrent negative control by at least 6 grains per nucleus, and the value for the negative control did not exceed 5. A dose response was not needed.

Test Conditions: Primary cultures of hepatocytes from livers of freshly perfused F344 rats were exposed to the test substance in the presence of ^{3}H-thymidine. Cytotoxicity was evaluated in a separate assay and used as a basis for dosage selection. The occurrence of UDS was visualized autoradiographically and quantified with the aid of microscopy.

A 10% solution of Pluronic® F68 Polyol in water was used to emulsify the test substance. This was diluted with medium so that the concentration of F68 in the dosing preparations was 2.5% (rangefinding) and 3.5% (UDS). Dosing preparations were added to the cultures in aliquots of 30 or 50 ul. This produced a culture concentration of 0.025 and 0.035% F68, respectively.

The positive control was 2-acetylaminofluorene (2-AAF) prepared using DMSO and Pluronic® F68 Polyol and administered at 0.2 ug/ml 2-AAF in the final culture.

The cells were grown in 3 ml (UDS) or 5 ml (rangefinding) Williams Medium E supplemented with 10% fetal bovine serum and insulin. Antibiotics were included. During the exposure period, 0.1M HEPES buffer, 2% by volume, and 0.1N HCL, 1% by volume, were present in the medium. The cells were cultured in plastic vessels. Incubation was in a carbon dioxide-enriched (5%), humidified atmosphere at 37°C. During the exposure period, cultures were sealed.

RANGEFINDING EXPERIMENT: In the rangefinding experiment, hepatocytes were harvested from one male rat aged 11 weeks and weighing 250 g. Two cultures each were prepared for the negative control, vehicle control and 8 levels of test substance. Approximately 1 x 10^5 cells/ml were seeded into each treatment culture and exposed to the test substance for 18 hours. The cells were then stained with trypan blue, fixed with formalin, and counted for viability determination. The culture vessel was taken as the experimental unit. The average number of viable cells per treatment group was determined. The relative viability was then calculated as the average number of viable cells in substance-treated cultures divided by that in the vehicle control cultures. For the evaluation of toxicity, at least 50% viability was desired. The final choice of treatment levels was based on the expectation that at least one level showed toxicity.

UDS EXPERIMENT: In the UDS experiment, hepatocytes were harvested from 1 male rat aged 13 weeks and weighing 270 g. Three cultures each were prepared for the negative control, vehicle control, positive control, and 4 levels of test substance. Approximately 1 x 10^5 cells/ml were seeded into each treatment culture and exposed to ^{3}H-thymidine and test substance for 18 hours. Cells growing on coverslips were rinsed, exposed to hypotonic solution, fixed, air dried and glued to microscope slides on Day 2. On Day 3, the slides were dipped in autoradiographic emulsion and stored in the dark at 2-8°C. Autoradiographs were developed, stained and coversliped on Day 13. The number of grains overlying each of 50 randomly selected nuclei per slide were counted microscopically. The highest of 3 cytoplasmic grain counts per cell was subtracted to obtain the net nuclear grain count. The individual slide was taken as the experimental unit. The average net nuclear grain count per slide (sum of net nuclear grain counts divided by 50) was calculated and the mean net nuclear grain count (average net nuclear grain count per slide divided by 3) was determined for each treatment level.

Results

Cytotoxic conc.:	2.048 mg/ml
Genotoxic effects:	Negative
Remarks:	In the rangefinding experiment, 1-hexene was toxic to primary hepatocytes at 2.048 mg/ml where 77% relative viability was observed following an 18-hr exposure period. At 5.0 mg/ml, the relative viability was 56.7%. In the UDS experiment, both positive and negative controls gave the expected responses. Due to toxicity at 3.5 and 5.0 mg/ml 1-hexene, only the 0.5 and 2.0 mg/ml levels were evaluated for UDS. Results for these two dose levels were negative for UDS.
Reliability:	(2) Reliable with restrictions: No confirmatory experiment was conducted.
References:	Gulf Life Sciences (1984) Hepatocyte Primary Culture/DNA Repair Test of GULFTENE 6, project #2071 (unpublished report).
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(2) **Test Substance**

Identity (*purity*): CAS No. 592-41-6, 1-Hexene (GULFTENE 6)

Method

Method/guideline:	Equivalent to USEPA TSCA 40 CFR 795.285 and ECC B21, except that the assay was not repeated.
Type:	BALB/3T3 Transformation Test
System of testing:	Non-bacterial
GLP:	No
Year:	1983
Cell line:	Mouse embryo cells, BALB/3T3-A31-1-1
Metabolic activation:	None
Concentrations tested:	Rangefinding experiment: 32, 64, 128, 256, 512, 1024, 2048 and 5000 ug/ml Transformation experiment: 256, 512, 1024, 2048 ug/ml
Statistical Methods:	A test was considered positive if there were: 1) a two-fold increase in Type-III foci at the highest dose over that seen in vehicle control cultures, with or without a dose-related response or 2) a two-fold increase at two or more consecutive dose levels. Where vehicle control cultures have no Type-III foci, at least 2 foci would be needed for a dose level to be considered positive.

Test Conditions: Cytotoxicity was evaluated in a separate assay and used as a basis for dosage selection.

A 10% solution of Pluronic®F68 Polyol in water was used to emulsify the test substance. This was diluted with medium so that the concentration of F68 in the dosing preparations was 2.5%. Dosing preparations were added to the cultures in aliquots of 50 ul. This produced a culture concentration of 0.025% F68. The positive control was 3-methylcholanthrene (3-MC) prepared using DMSO and Pluronic® F68 Polyol and administered at 1 ug/ml 3-MC in the final culture.

The cells were received at Passage 14 after origination of the subclone. The cells were subcultured once, tested for presence of adventitious infectious agents and for capability to respond to known transforming agents, and frozen. Cultures used in testing were less than 4 additional passages from frozen stock. The cells were grown in 5 ml Eagle's Minimum Essential Medium supplemented with 10% heat-inactivated fetal calf serum. Antibiotics were included. During the exposure period, 1M HEPES buffer, 2% by volume, was present in the medium. The cells were cultured in glass vessels. Incubation was in a carbon dioxide-enriched (5%), humidified atmosphere at 37°C. During the exposure period only, cultures were sealed and placed in a vented 37°C incubator without carbon dioxide enrichment or humidified atmosphere.

RANGEFINDING EXPERIMENT: Each treatment group (medium, vehicle, and 8 levels of test substance) consisted of two cultures. Approximately 1 x 10^4 cells were seeded into each treatment flask on Day 1. The cultures were exposed to the test substance for 2 days, beginning on Day 2, then trypsinized and counted on Day 4 with a Coulter Model ZB cell counter. The culture vessel was taken as the experimental unit. The average number of surviving cells per treatment group was determined. The relative survival was then calculated as the average number of surviving cells in substance-treated cultures divided by that in the vehicle control cultures. For the evaluation of toxicity, at least 20% survival was desired. The final choice of treatment levels was based on the expectation that at least one level showed toxicity.

TRANSFORMATION EXPERIMENT: Each group (medium control, vehicle control, positive control, and 4 levels of test substance) consisted of 15 flask cultures for transformation and 2 flask cultures for cloning. Transformation flasks were seeded with approximately 1 x 10^4 cells and cloning flasks with approximately 100 cells on Day 1. The cells were exposed to test substance for 2 days beginning on Day 2. The medium was changed on all cultures on Day 4. Cloning cultures were fixed

and stained for colony counting on Day 8. Colonies (at least 50 cells) were counted visually and, where required, examined microscopically. The medium was changed weekly on all transformation flask cultures. Fixation and staining of flask cultures for focus counting and evaluation were on day 29. Foci were counted visually and examined microscopically to determine type.

The cloning efficiency was determined by dividing the average number of colonies (at least 50 cells) per flask by the number of cells seeded and converting to a percent. The relative cloning efficiency was determined by dividing the cloning efficiency for each treatment group by the cloning efficiency for the vehicle control, and converting to a percent. The transformation frequency for each group was the total number of Type III foci divided by the total number of flasks per group.

Results:

Cytotoxic conc.: Concentrations of 32-5000 ug/ml were cytotoxic

Genotoxic effects: Negative

Remarks: In the rangefinding experiment, 1-hexene was toxic to BALB/3T3 cells at 32 ug/ml when 67.6% viability was observed following a 3-day exposure period. Viability decreased slightly to 54.8% at 1024 ug/ml, then decreased sharply to 24.1% at 2048 ug/ml, and finally to 4% at 5000 ug/ml. In the transformation experiment, cloning efficiency was used as a measure of toxicity. Toxicity became evident at 2048 ug/ml (57.9% relative cloning efficiency). The positive control gave the expected response. The negative controls were within acceptable limits for the test. No treatment level exceeded the negative controls for Type III foci. Under the conditions of the test, 1-hexene was negative for cell transformation.

Reliability: (2) Reliable with restrictions: There was no repeat experiment.

References: Goode, J.W. and Brecher, S. (1983) GULFTENE 6: BALB/3T3 transformation test, Project 2072. Sponsored by Gulf Life Sciences Institute, Pittsburg, PA (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(3) Test Substance

Identity (*purity*): CAS# 558-37-2, Neohexene (3,3-dimethylbutene-1, 98.5% purity).

Method

Method/guideline:	OECD 479
Type:	*In vitro* sister chromatid exchange (SCE) assay in Chinese hamster ovary cells
System of testing:	non- bacterial
GLP:	Not specified
Year:	1988
Cell line:	Chinese Hamster Ovary (CHO) cells
Metabolic activation:	With and without S9 fraction of livers from Sprague-Dawley rats pretreated with Aroclor 1254
Concentrations tested:	0, 1.3, 4.4, 13.2, 44, and 132 µg/ml
Statistical Methods:	Not specified
Test Conditions:	Solvent controls: dimethylsulfoxide (DMSO). Positive controls: ethylmethanesulfonate (without S9), cylcophosphamide (with S9). The test substance was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) both in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9. The test included concurrent solvent and positive controls and five doses of the test substance. The test substance was soluble in the solvent (DMSO) at 100 mg/ml. The maximum dose selected was 132 µg/plate based on observed growth inhibition in an initial toxicity study. Duplicate cultures were prepared for all dose levels and controls. Cells were exposed to the test substance for 2 hours, washed twice, and BrdU added to each culture. Cells were sampled 24 hours after BrdU addition; colcemid was added 2 hours prior to fixation. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Results

Cytotoxic conc.:	132 µg/plate in the initial cytotoxicity study.
Genotoxic effects:	Negative with and without metabolic activation.
Remarks:	No increases in SCEs were noted in cultured CHO cells treated with the test substance, with or without S9.
Reliability:	(1) Reliable without restrictions
References:	Hazleton Laboratories America, Inc. (1982). Neohexene: *In vitro* sister chromatid exchange assay in Chinese hamster ovary cells. Conducted for Phillips Petroleum Company (unpublished report).

5.7 Genetic Toxicity *in vivo*

A. Test Substance

Identity (purity): CAS No. 68526-52-3, Alkenes, C6

Method

Method/guideline:	EPA OTS 798.5395
Type:	Micronucleus Assay
GLP:	Yes
Year:	1993
Species:	Mouse
Strain:	B6C3F1
Sex:	Male and female
Route of Administration:	Inhalation – saturated vapor
Concentration levels:	Target exposure of test substance: 1000 ppm; Actual mean exposure: 1057 ppm (3638 mg/m^3) (saturated vapors, no aerosol).
Exposure period:	6 hours/day for 2 consecutive days
Statistical methods:	To determine the percentage of micronuclei, 1000 polychromatic erythrocytes from each animal were examined for micronuclei. To determine the percentage of polychromatic erythrocytes, the number of polychromatic erythrocytes in a total of 1000 erythrocytes was determined. Statistical analysis included calculation of means and standard deviations of the micronuclei data and a test of equality of group means by a standard one way analysis of variance at each time period. When the ANOVA was significant, comparisons of carrier control to dosed group means were made according to Duncan's Multiple Range Test. Data from both males and females were analyzed as a single group to facilitate comparisons to published data.

Test Conditions: For the purpose of this study, the test material was considered to be free of impurities. Vapors were generated by forcing the test material with a piston pump through a glass cylinder with heating tape. Vapors were drawn into the chamber with air flow at a rate of 200 liters/minute. Nominal and actual concentrations were determined by net weight loss of the test material and by gas chromatography, respectively. Animals were approximately 8 to 10 weeks old at initiation of the study. Five animals/sex were exposed to vapors of the test substance for 6 hours per day on 2 consecutive days. During each exposure, animals were observed hourly. The positive control, cyclophosphamide in water, was administered by oral gavage as a single dose of 40 mg/kg to 5 animals/sex. The negative controls (five animals/sex) received a sham exposure of air. Animals from the treated and negative control groups were sacrificed by carbon dioxide asphyxiation at appropriately 24 hours

after the second day of exposure. Animals treated with cyclophosphamide were sacrificed 24 hours following dose administration. Immediately upon sacrifice, the bone marrow was removed from both femurs of each animal, resuspended, and prepared for microscopy. Samples were blindly coded and stained with acridine orange.

Results

Effect on PCE/NCE ratio:	None
Genotoxic effects:	Negative
NOEL:	1057 ppm (3.638 mg/L) (saturated vapor)
Remarks:	The test material was not clastogenic since it did not induce a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes, indicating that the test substance is not clastogenic. In addition, the test substance did not induce a statistically significant decrease in the mean percent of polychromatic erythrocytes, indicating that the test substance did not induce bone marrow toxicity. The positive control did induce a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes and was therefore clastogenic. The sham control values for the mean number of micronucleated polychromatic erythrocytes were within the normal range for the negative control. Under the conditions of this assay, Alkenes, C6 are not clastogenic following inhalation exposure in mice.
Reliability:	(1) Reliable without restrictions
Flag:	Key study for SIDS endpoint
References:	Exxon Biomedical Sciences, Inc. (1991c) Alkenes, C6: In vivo mammalian bone marrow micronucleus assay: inhalation dosing method (unpublished report).

B. Test Substance

Identity (purity): CAS No. 68526-52-3, Alkenes, C6

Method

Method/guideline:	EPA OTS 798.5395
Type:	Micronucleus Assay
GLP:	Yes
Year:	1991
Species:	Mouse
Strain:	B6C3F1
Sex:	Male and female
Route of Administration:	Oral gavage

Concentration levels: 1.25, 2.5, and 5 g/kg. Concentrations were based on the results of a range-finding study.

Exposure period: Single dose

Statistical methods: To determine the percentage of micronuclei, 1000 polychromatic erythrocytes from each animal were examined for micronuclei. To determine the percentage of polychromatic erythrocytes, the number of polychromatic erythrocytes in a total of 1000 erythrocytes was determined. Statistical analysis included calculation of means and standard deviations of the micronuclei data and a test of equality of group means by a standard one way analysis of variance at each time period. When the ANOVA was significant, comparisons of carrier control to dosed group means were made according to Duncan's Multiple Range Test. A standard regression analysis was performed to test for a dose response. Sexes were analyzed separately.

Test Conditions: Animals were approximately 7 to 8 weeks old at initiation of the study. The test material and the carrier (corn oil) were administered by oral gavage as a single dose to 15 mice per sex per dose (not fasted). For the purpose of this study, the test material was considered to be free of impurities. The positive control, cyclophosphamide, was administered by intraperitoneal injection as a single dose of 40 mg/kg. Animals from the appropriate groups (5 animals/sex/group) were sacrificed by carbon dioxide asphyxiation at appropriately 24, 48 and 72 hours after dose administration. Animals dosed with cyclophosphamide were sacrificed at 24 hours only. Immediately upon sacrifice, the bone marrow was removed from both femurs of each animal, resuspended, and prepared for microscopy. Samples were blindly coded and stained with acridine orange.

GLP Deviations: Analysis of the material stability and purity were the responsibility of the study sponsor, it is not known whether these procedures were performed.

Results

Effect on PCE/NCE ratio: None

Genotoxic effects: Under the conditions of this study, Alkenes, C6 were clastogenic to the bone marrow of B6C3F1 mice when administered by oral gavage at 5.0 g/kg 24 hours prior to analysis, but not at 48 and 72 hours post-exposure.

NOEL: 2.5 g/kg

Remarks: The test material induced a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes per 1000 cells at 5.0 g/kg for the 24-hour males and females (6.8 +/- 3.12 and 5.4 +/- 2.1, respectively). The mean number of micronucleated polychromatic erythrocytes for the positive controls at 24 hours for males and females were 36.2 +/- 10.5 and 30.4 +/- 9.0 and the negative controls were 2.4 +/- 0.9 and 2.6 +/- 1.5. The increase in micronucleated polychromatic erythrocytes observed at 24 hours was dose-related. However, at 48 and

72 hours after the initial exposure, the mean number of micronuclei did not differ between the control and treated groups. The test substance did not induce a statistically significant decrease in the mean percent of polychromatic erythrocytes, indicating that the test substance is not toxic to bone marrow. The positive control induced significant increases in the mean number of micronucleated polychromatic erythrocytes. The positive control also induced a statistically significant decrease in the mean percent of micronucleated polychromatic erythrocytes in male mice. Carrier control values for the mean percent of micronucleated polychromatic erythrocytes and the mean number of micronucleated polychromatic erythrocytes were within the normal range for the negative controls.

Alkenes, C6 produced a slight, transient increase in micronucleated polychromatic erythrocytes at the highest level by oral gavage. However, given that inhalation is the primary route of industrial exposure, a micronucleus study was repeated with inhalation as the route of administration. This study produced negative results (Section 5.7.A). In addition, Alkenes, C6 are not mutagenic *in vitro*. Collectively, these data suggest that Alkenes, C6 are not expected to be genotoxic.

Reliability: (1) Reliable without restrictions

Flag: Key study for SIDS endpoint

References: Exxon Biomedical Sciences, Inc. (1991b) Alkenes, C6: In vivo Mammalian Bone Marrow Micronucleus Assay: Oral Gavage Method (unpublished report).

C. Test Substance

Identity (purity): CAS No. 592-41-6, 1-Hexene (GULFTENE 6)

Method

Method/guideline:	OECD 474
Type:	Micronucleus Assay
GLP:	Yes
Year:	1983
Species:	Mouse
Strain:	Crl:CD-1 (ICR)BR
Sex:	Male and female
Route of Administration:	Inhalation – vapor
Concentration levels:	Target exposure of test substance: 1000, 10,000, 25,000 ppm (3442, 34,421, 86,053 mg/m^3); Actual mean exposure (TWA): Day 1 = 991, 10758, 25302 ppm (3.4, 37, 87 g/m^3); Day 2 = 1244, 10272, 23271ppm (4.3, 35, 80 g/m^3)

Exposure period: 2 hours/day for 2 consecutive days

Statistical methods: Statistical analysis included calculation of means and standard deviations of the body weight and micronuclei data and a test of equality of group means by a student's t-test at each time period. The data were also compared to those in pertinent historical data files. Data from males and females were analyzed separately. The test would be considered positive if there were a significant increase in micronucleated PCEs at any dose level and if a dose-related response were evident.

Test Conditions: The test substance was aerosolized with a ball-jet nebulizer using clean, filtered air. To achieve 1000 ppm, air was passed over the liquid surface and the resultant vapor diluted further with air. To achieve 10,000 and 25,000 ppm, the test substance was drawn through a submerged feeding tube to the nebulizer jet. The released vapor was diluted with air as needed. The negative control group received only clean, filtered air. The chambers were sampled before and during the treatment interval. Nominal and actual concentrations were determined by net weight loss of the test material and by gas chromatography, respectively. Particle size was determined once daily for each exposure chamber with an aerodynamic particle sizer. Results indicate that the particulate matter was less than 1% by weight of the chamber atmosphere.

Mice (45 each) were 12 weeks of age and weighed 35-43 g (males) and 26-33 g (females) at the start of treatment. Test substance and the sham air negative control were administered to 10 animals/sex/per group on Day 1 and 2. The positive control, cyclophosphamide (7.5 mg/ml in 0.9% sodium chloride), was administered by intraperitoneal injection as a single dose of 75 mg/kg to 5 animals/sex on Day 1. Animals were weighed on Days 1, 3, and 4 and observed daily. Animals from each group were sacrificed on Day 3 and 4 except that animals given cyclophosphamide were sacrificed only on Day 3. Immediately after sacrifice, bone marrow smears were prepared. Samples were stained with May-Grunwald and Giemsa stains and examined microscopically. To determine the percentage of micronuclei, 1000 PCE from each animal were examined for micronuclei. To determine the percentage of PCE, the number of PCE in a total of 1000 erythrocytes was determined.

Results

Effect on PCE/NCE ratio: An equivocal decrease in the ratio of PCE to normochromatic erythrocytes (NCE) was observed in test substance treated females at all dose levels on Day 3 (PCE/NCE = 1.6, 1.2, 0.9, 1.1 for 0, 1000, 10000, 25000 ppm, respectively)

Genotoxic effects: Negative

NOEL: 25,000 ppm (86,053 mg/m^3) (nominal, vapor)

Remarks: One male mouse died on Day 3 due to wounds received from another male. Treatment-related findings were lethargy and rapid respiration

during exposure to 10,000 and 25,000 ppm. Recovery was rapid when the mice were returned to an air atmosphere. There was no treatment-related change in body weight.

The test material did not induce a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes, indicating that the test substance is not clastogenic. The positive control did induce a statistically significant increase in the mean number of micronucleated polychromatic erythrocytes.

Reliability: (1) Reliable without restrictions

References: Gulf Life Sciences (1983) Micronucleus Test in Mouse Bone Marrow: GULFTENE 6 Administered by Inhalation Using 2 Daily 2-Hour Treatments, project #82-119 (unpublished report).

Other: This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

5.8 Carcinogenicity

No data available

5.9 Reproductive Toxicity (including Fertility and Developmental Toxicity).

A. Fertility

(1) Test Substance

Identity:	CAS No. 68526-52-3, Alkenes C6, internal branched
Remarks:	Composition: C5 n-olefins = 0.5%, C5 iso-olefins = 1.3%, C6 n-olefins = 10.4%, C6 iso-olefins = 55.6%, C5 n-paraffins = 3.3%, C5 iso-paraffins = 9.3%, C6 iso-paraffins = 17.8%, C7 iso-olefins = 1.0%

Method

Method/guideline:	OECD 422
Test type:	Combined repeated dose toxicity study with reproduction/developmental toxicity screening test
GLP:	Yes
Year:	2002
Species:	Rat
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR
Route of Administration:	Oral gavage
Concentration levels:	0, 100, 500 or 1000 mg/kg b.w./day
Sex:	Males and females

Control group and treatment:	Concurrent vehicle control (corn oil)
Frequency of treatment:	Daily
Duration of test:	Up to 53 days (reproduction phase), see Remarks
Premating exposure period for males:	14 days
Premating exposure period for females:	14 days
Statistical methods:	Data for the reproduction/developmental screening study, including body weights, body weight gain, food consumption and mean live litter size were analyzed by One-Way Analysis of Variance (ANOVA). If significance was detected, control to treatment group comparisons were performed using Dunnett's test. Count data were analyzed using R x C Chi-Square test followed by Fishers Exact Test for copulation and fertility indices, pup sex ratios, the number of live and dead pups per group (on lactation day 0) and pup survival (after lactation day 0). All analyses were two-tailed with a minimum significance level of 5% ($p<0.05$).

Test Conditions: This study was conducted to: (1) provide screening information on the repeated-dose systemic toxicity of the test substance, with emphasis on potential neurological effects, and (2) serve as a screening study for potential reproductive and developmental effects in male and female rats.

REPRODUCTIVE TOXICITY PHASE (see Section 5.5.A for the test conditions for general toxicity phase, including information for reproductive toxicity phase males): On the day following receipt, animals were approximately 9 wks of age and weighed 237 – 296 g (males) and 183 - 234 g (females). Animals were acclimated for 17 days prior to dosing. The study consisted of one control group and three treatment groups with 12 animals in each group. Animals were dosed for a minimum of 14 days prior to mating and continuing through lactation day 3. Detailed clinical observations were performed a minimum of weekly until evidence of mating was detected, daily during gestation and lactation and on the day of scheduled euthanasia. Individual body weights were recorded on days 0, 3, 7 and 12 prior to mating. When positive evidence of mating was detected, the females were weighed on gestation days 0, 7, 14, and 20. Following parturition, the females were weighed on lactation days 1 and 4. Females without evidence of mating were weighed twice weekly until euthanasia. Individual food consumption was recorded on the same days as body weights (except during cohabitation). Following a minimum of 14 days of treatment, each female was cohabited with a single male from the same treatment group (1:1 pairing) in the toxicity study. Each mating pair was observed daily for evidence of copulation. Evidence of mating was determined by the presence of a copulatory plug in

the vagina or a sperm positive vaginal smear. The day evidence of copulation was confirmed was designated as day 0 of gestation and the female was returned to her cage. If no evidence of copulation was observed after 14 days of mating, the female was separated from the male and the mating phase was concluded. On gestation day 18, females with confirmed copulation were transferred to individual nesting boxes. The females and their offspring remained together until lactation day 4. All animals were subjected to a complete gross necropsy examination at the time of death or euthanasia (lactation day 4 or post-breeding period day 25). Females with total litter loss were euthanized on the day that no surviving pups remained. Organ weights (liver, kidneys, adrenals, thymus, spleen, brain and heart) were recorded from surviving animals and the following tissues and organs were preserved from all animals: all gross lesions, accessory genital organs, adrenals, aorta, brain, cecum, colon, duodenum, esophagus, exorbital lachrymal glands, eyes with optic nerve, femur, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, mesenteric/mandibular/ mediastinal lymph nodes, ovaries, pancreas, peripheral nerve, pituitary, rectum, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, submaxillary salivary gland, testes, thymus, thyroid, parathyroid, tongue, trachea, and urinary bladder. All tissues and organs collected at necropsy from males in the control and high-dose groups and animals found dead, gross lesions from males in the low- and mid-dose groups, and all gross lesions in F0 females were examined microscopically. The following parameters were recorded for each pup during lactation: viability (daily from days 0 – 4), external examinations and sex determinations (days 0 and 4); and body weights (days 1 and 4). Pups that were stillborn or died were subjected to a gross necropsy examination, with emphasis on developmental morphology. All internal gross lesions (except atelectasis; and scabbing, subcutaneous hemorrhage or other lesions resulting from apparent cannibalism) were preserved. All surviving pups were euthanized on lactation day 4 and examined macroscopically for structural abnormalities or other pathological changes. All gross lesions were preserved.

Results

NOAEL (NOEL): NOAEL (reproductive toxicity, paternal and F1) = 1000 mg/kg/day (study author assigned)

Actual dose received by dose level by sex if known: As administered. Analysis of dosing mixtures confirmed that mixtures were accurately prepared.

Remarks: No test article-related mortality occurred in males or F0 females. One F0 female in the 500 mg/kg/day group was found dead on

lactation day 0. No adverse clinical signs were observed for this female prior to death. One other F0 female in the 500 mg/kg/day group was euthanized due to total litter loss on lactation day 2, the day all pups in the litter were found dead. This female had apparent dystocia. The animal appeared to have a blockage in the vaginal area; slight pressure was applied and 2 pups were delivered. The female delivered a total of 17 pups (2 live and 15 dead). Clinical signs including eyelids partially closed, eyes and skin pale in color and labored breathing were noted for this female following the onset of parturition. Two females in the 100 mg/kg/day group and one female in the 1000 mg/kg/day group with no evidence of mating were euthanized on post-breeding period day 25. No remarkable clinical signs were observed for these females. All other females survived to scheduled euthanasia on lactation day 4. Most clinical signs observed for the surviving F0 females were generally unremarkable and did not appear to follow any dose response pattern. However, a low incidence of post-dose salivation was seen in 4 of 12 females in the 1000 mg/kg/day group. Post-dose salivation was observed for males (11/12) in the 1000 mg/kg/day group. Remarkable clinical signs in males in the 500 mg/kg/day group were limited to a single incidence of post-dose salivation in 1 male. No remarkable clinical signs were observed for males in the 100 mg/kg/day group.

There were no statistically significant or toxicologically meaningful differences noted in mean body weights or body weight gain for males or for F0 females, or food consumption for males. Mean food consumption of F0 females in the 1000 mg/kg/day group was statistically higher than controls during gestation days 14-20. This difference was not considered toxicologically meaningful since it did not follow a consistent pattern and was an increase rather than a decrease.

The F0 female mating index was 100%, 83.3%, 100% and 91.7% in the 0, 100, 500 and 1000 mg/kg/day groups, respectively. The F0 female fertility index was 100% in the control and test article-treated groups. The F0 mean gestation length was 21.9, 22.0, 21.8 and 21.7 days in the 0, 100, 500 and 1000 mg/kg/day groups, respectively. Totals of 12, 10, 12 and 11 F0 females in the 0, 100, 500 and 1000 mg/kg/day groups, respectively, completed delivery.

The mean number of F1 pups delivered and the live birth index were comparable between the control and test substance-treated groups. However, the viability index of pups in the 500 mg/kg/day group was statistically lower than controls. This difference was not considered toxicologically meaningful since a similar difference was not noted for pups in the 1000 mg/kg/day group. The mean number of implantation sites and mean number of corpora lutea were comparable between the control and test

substance-treated groups. The mean live pups per litter and the pup sex ratio were comparable between the control and test substance-treated groups on lactation days 0 and 4. Mean pup weights were slightly but not statistically lower than controls in the 500 mg/kg/day group on lactation day 1 (6.8 g) and in the 1000 mg/kg/day group on lactation days 1 and 4 (6.7 and 9.3 g, respectively). However, the mean body weights in these groups were within the range of the laboratory's historical control data (i.e., 6.5-7.5 g on lactation day 1 and 8.5-11.1 g on lactation day 4). Mean pup weights in the 100 mg/kg/day group were comparable to controls on lactation days 1 and 4.

No remarkable gross necropsy findings were noted for surviving F0 females in the control or test substance-treated groups, and there were no toxicologically meaningful differences in absolute or relative organ weights between the groups. No toxicologically meaningful microscopic findings were noted for F0 females in the treated groups.

(See Section 5.5.A for additional general toxicity results for males and females; and Section 5.9.B(1) for developmental toxicity results.)

Reliability: (1) Reliable without restrictions.

Flag: Key study for SIDS endpoint.

References: Thorsrud, B.A. (2003) A combined repeated dose toxicity study and reproduction/developmental screening study in Sprague Dawley rats with (C6) alkenes, Study No. 3604.2, Springborn Laboratories, Inc., Ohio Research Center, Spencerville, Ohio; conducted for American Chemistry Council (Higher Olefins Panel) (unpublished report).

(2) Test Substance

Identity (purity): CAS No. 592-41-6, 1-Hexene

Remarks: Three test articles were blended to produce the final test article consisting of 90-100% 1-hexene (NEODENE 6, GULFTENE 6 and alpha olefin 6).

Method

Method/guideline: OECD 421 (modified) (see Sec. 5.5.D for general toxicity endpoints)
Type: Reproduction/Developmental Toxicity Screening Study
GLP: Yes
Year: 1995
Species: Rat

Strain:	Sprague-Dawley
Route of administration:	Oral gavage
Concentration levels:	0, 100, 500, 1000 mg/kg/day
Sex:	Male and female
Control group and treatment:	Corn oil by oral gavage
Frequency of treatment:	Daily
Duration of test:	Males: 44 days; females: 41-55 days
Premating exposure period for males:	28 days
Premating exposure period for females:	14 days
Statistical methods:	Continuous data, including body weights, body weight gain, feed consumption, organ weights, pup body weights, gestation length, mean live litter size and implantation scar counts were analyzed by using a One-Way analysis of Variance. If significance [$P<0.05$] was detected, group by group comparisons were performed using Dunnett's test. Count data were analyzed utilizing Chi-Square test for copulation and fertility indices, pup sex ratios, the number of live and dead pups per group on lactation day 0 and pub survival after lactation day 0. All analyses utilized two-tailed tests for a minimum significance level of 5% comparing the control to the treated groups.

Test Conditions: 12 male rats (195-242 g, 6 weeks old) per group were exposed for 28 days prior to mating, and through mating until euthanasia for a total of 44 consecutive days of dosing; 12 females (163-219 g, 8 weeks old) per group were dosed for 14 days prior to mating, during mating, gestation and lactation through euthanasia at lactation day 4 [41-55 consecutive days]. Dose levels were 0, 100, 500, 1000 mg/kg/day in a corn oil vehicle [5 mL/kg]. Viability and development of the pups were followed through lactation day 4. Animals were observed daily for clinical signs of toxicity. Body weights and food consumption were determined weekly. Each male was cohabited with one female and observed daily for evidence of copulation. If no evidence of copulation was confirmed after 10 days of cohabitation, the female was separated from the first male and placed with a second (proven) male for a maximum of 5 days. Females that delivered were necropsied on lactation day 4. Females that failed to deliver were necropsied 25 days after evidence of mating was detected. For females, the number of uterine implantation scars was recorded and the ovaries and brain were weighed. For males, after 43 days of dosing, the viscera were examined, and brain, testes and epididymides weighed. The ovaries, testes, epididymides, liver, kidneys, and peripheral

(sciatic) nerve of control and high dose animals, the kidneys of the low and mid dose animals, and all gross lesions from each group, were processed for microscopic examination. Pup viability, weight, and a detailed examination of the pups including sex determination, were performed on lactation days 0 and 4. All intact pups dying prior to lactation day 4 were necropsied and examined with emphasis on developmental morphology.

Results

NOEL:	NOEL for P generation (reproductive effects) >1000 mg/kg; NOEL for F1 generation > 1000 mg/kg/day
Actual dose received by dose level by sex if known:	As administered. Analysis of dosing mixtures confirmed that mixtures were accurately prepared.
Maternal and Paternal general toxicity:	see Sec. 5.5.D
Reproductive toxicity observed in parental animals:	none
Reproductive toxicity observed in offspring:	none
Remarks:	There was no evidence of impaired reproductive capabilities in the F0 generation, as measured by effects on copulation and fertility, precoital intervals, gestation length, time to delivery or unusual nesting behaviour.
Reliability:	(1) Reliable without restrictions
Flag:	Key study for SIDS endpoint
References:	Gingell, R., Daniel, E.M., Machado, M, and Bevan, C. (2000) Reproduction/developmental toxicity screening test in rats with orally-administered 1-Hexene. Drug and Chem. Toxicology 23(2)327-338.
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(3) Test Substance

Identity (purity):	CAS No. 592-41-6, 1-Hexene (99+%, NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 413 (see Section 5.5.B for general toxicity endpoints)
Type:	90-day subchronic inhalation toxicity study
GLP:	Yes
Year:	1984
Species:	Rat
Strain:	F344
Route of administration:	Inhalation
Concentration levels:	0, 300, 1000, 3000 ppm (0, 1033, 3442, 10,326 mg/m^3)
Sex:	Males and females
Control group and treatment:	Air exposed
Freq. of treatment:	6 hr/day, 5 days/week, 13 weeks
Duration of test:	13 weeks
Statistical methods:	Unadjusted body weights were analyzed by Dunnet's test. Organ weights, clinical chemistry, hematology, urinalysis, and organ-to-body weight ratio data were analyzed by Dunnett's t-test on ranked data. The Rotorod data for neuromuscular coordination were adjusted by summing the time for the best 3 of 4 trials for each rat.

Test Conditions: The objective of this study was to evaluate the toxicity of 1-hexene following repeated inhalation exposures in male and female Fischer 344 rats. Groups of young 40 male and 40 female rats (125-160 g at study initiation) were exposed for 6 hours per day, 5 days per week, over a 13-week period. Treatment groups (10 rats/sex/group) consisted of air-exposed control (0 ppm) and three test groups of 300, 1000, and 3000 ppm 1-hexene. During the treatment period, the rats were observed daily for clinical signs of toxicity; body weights were measured at 7-day intervals. After 7 weeks of exposure and at the end of the treatment period, the rats were subject to macroscopic and microscopic pathology, clinical chemistry, hematology, urinalysis, and sperm counts. Reproductive organ examined at necropsy: right testicle without epididymides. Reproductive organs examined microscopically: right testicle and epididymides. (See Section 5.5.B for details for general toxicity endpoints.). The left testicle from each male rat in the main study at the interim and final sacrifice was used for sperm enumeration. The testis was detunicated, weighed, homogenized and sonicated in distilled water, and aliquots of homogenate were removed for sperm head count determination. Counts were performed with a hemocytometer and phase-contrast microscopy.

Results

NOAEL:	The NOEL for reproductive effects from the limited data for reproductive organs and sperm counts in the 90-day study appears to be at the mid-concentration of 1000 ppm.
Actual dose received by dose level by sex:	0, 300, 1000, 3000 ppm
Remarks:	Please see Repeated Dose Toxicity Section 5.5.B for general toxicity results. At terminal sacrifice, there appeared to be a dose-related increase in testes weight which was statistically significant at the highest exposure concentration of 3000 ppm (see page 189 of the original study); however, when the left testicle was detunicated prior to weighing, there was no statistically significant increase in testis weight compared with the controls. This increase in testes weight did not appear to be accompanied by any histopathology nor any apparent effect on sperm count. Sperm morphology and motility were not evaluated. An increase in sperm counts and testis weight in all animals at terminal sacrifice was observed when compared to the interim sacrifice (page 121 vs 189 in the original study), however, the increase was considered to be attributed to the increase in age of the animal. Since testes weight measurements in and of themselves do not indicate the exact nature of an effect, a significant increase or decrease is indicative of an adverse effect. In this case, there was no histopathology and the sperm counts were within control range. However, damage to the testes may be detected as a weight change only at doses higher than those required to produce significant effects in other measures of gonadal status and it appears that only a minimal evaluation of gonadal status was conducted in this 90-day study. As a result the significance of increases in testes weight is unclear.
Reliability:	(1) Reliable without restrictions
References:	Gingell R, Bennick JE, and Malley LA., 1999. Subchronic inhalation study of 1-hexene in Fischer 344 rats Drug Chem Toxicol. 22(3):507-28. [USEPA review of unpublished Shell Development Company report (90-Day Vapor Inhalation Study in Rats with NEODENE 6 Alpha Olefin, WTP-207, 1984) conducted by Katherine Anitole [8/26/99.]

Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

B. Developmental Toxicity

(1) Test Substance

Identity:	CAS No. 68526-52-3, Alkenes C6, internal branched
Remarks:	Composition: C5 n-olefins = 0.5%, C5 iso-olefins = 1.3%, C6 n-olefins = 10.4%, C6 iso-olefins = 55.6%, C5 n-paraffins = 3.3%, C5 iso-paraffins = 9.3%, C6 iso-paraffins = 17.8%, C7 iso-olefins = 1.0%

Method

Method/guideline:	OECD 422
Test type:	Combined repeated dose toxicity study with reproduction/developmental toxicity screening test
GLP:	Yes
Year:	2002
Species:	Rat
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR
Route of Administration:	Oral gavage
Concentration levels:	0, 100, 500, or 1000 mg/kg b.w./day
Sex:	Males and females
Control group and treatment:	Concurrent vehicle control (corn oil)
Frequency of treatment:	Daily
Duration of test:	Up to 53 days (reproduction phase), see Remarks
Premating exposure period for males:	14 days
Premating exposure period for females:	14 days
Statistical methods:	Data for the reproduction/developmental screening study, including body weights, body weight gain, food consumption and mean live litter size were analyzed by One-Way Analysis of Variance (ANOVA). If significance was detected, control to treatment group comparisons were performed using Dunnett's test. Count data were analyzed using R x C Chi-Square test followed by Fishers Exact Test for copulation and fertility indices, pup sex ratios, the number of live and dead pups per group (on lactation day 0) and pup survival (after lactation day 0). All analyses were two-tailed with a minimum significance level of 5% ($p<0.05$).
Test Conditions:	This study was conducted to: (1) provide screening information on the repeated-dose systemic toxicity of the test substance, with emphasis on potential neurological effects, and (2) serve as a

screening study for potential reproductive and developmental effects in male and female rats.

REPRODUCTIVE TOXICITY PHASE: See Section 5.5.A for test conditions for the general toxicity phase, and Section 5.9.A(1) for test conditions for the reproductive toxicity phase.

Results

NOAEL (NOEL): NOAEL (maternal and developmental toxicity) = 1000 mg/kg/day (study author assigned)

Actual dose received by dose level by sex if known: As administered. Analysis of dosing mixtures confirmed that mixtures were accurately prepared.

Remarks: See Section 5.5A for general toxicity results, and Section 5.9.A(1) for additional reproductive toxicity results.

The mean number of F1 pups delivered and the live birth index were comparable between the control and test substance-treated groups. However, the viability index of pups in the 500 mg/kg/day group was statistically lower than controls. This difference was not considered toxicologically meaningful since a similar difference was not noted for pups in the 1000 mg/kg/day group. The mean number of implantation sites and mean number of corpora lutea were comparable between the control and test substance-treated groups. The mean live pups per litter and the pup sex ratio were comparable between the control and test substance-treated groups on lactation days 0 and 4. Mean pup weights were slightly but not statistically lower than controls in the 500 mg/kg/day group on lactation day 1 (6.8 g) and in the 1000 mg/kg/day group on lactation days 1 and 4 (6.7 and 9.3 g, respectively). However, the mean body weights in these groups were within the range of the laboratory's historical control data (i.e., 6.5-7.5 g on lactation day 1 and 8.5-11.1 g on lactation day 4). Mean pup weights in the 100 mg/kg/day group were comparable to controls on lactation days 1 and 4.

There were no toxicologically meaningful differences in pup observations during lactation. There appeared to be a slight increase in the incidence of subcutaneous hemorrhage(s) and pups small in size (qualitative measurement) for F1 pups in the 1000 mg/kg/day group during lactation days 0-4. However, these changes were not considered toxicologically meaningful since the subcutaneous hemorrhages were distributed over many parts of the body and the pup body weights were within the historical control range. No other remarkable findings were noted in the pups during lactation.

No remarkable gross necropsy findings were noted for pups found dead during the study or euthanized at study termination. A low incidence of commonly occurring findings was noted sporadically throughout the groups; but, none of the findings followed a consistent pattern or dose response.

Reliability: (1) Reliable without restrictions.

Flag: Key study for SIDS endpoint.

References: Thorsrud, B.A. (2003) A combined repeated dose toxicity study and reproduction/developmental screening study in Sprague Dawley rats with (C6) alkenes, Study No. 3604.2, Springborn Laboratories, Inc., Ohio Research Center, Spencerville, Ohio; conducted for American Chemistry Council (Higher Olefins Panel) (unpublished report).

(2) **Test Substance**

Identity (purity): CAS No. 592-41-6, 1-Hexene

Remarks: Three test articles were blended to produce the final test article consisting of 90-100% 1- hexene (NEODENE 6, GULFTENE 6 and alpha olefin 6).

Method

Method/guideline:	0ECD 421 (modified)
Type:	Reproduction/Developmental Toxicity Screening Study
GLP:	Yes
Year:	1995
Species:	Rat
Strain:	Sprague-Dawley
Route of administration:	Oral gavage
Concentration levels:	0, 100, 500, 1000 mg/kg/day
Sex:	Male and female
Control group and treatment:	Corn oil by oral gavage
Frequency of treatment:	Daily
Duration of test:	Males: 44 days; females: 41-55 days
Premating exposure period for males:	28 days
Premating exposure period for females:	14 days
Statistical methods:	Continuous data, including body weights, body weight gain, feed consumption, organ weights, pup body weights, gestation length, mean live litter size and implantation scar counts were analyzed by using a One-Way analysis of Variance. If significance [P<0.05] was detected, group by group comparisons were

performed using Dunnett's test. Count data were analyzed utilizing Chi-Square test for copulation and fertility indices, pup sex ratios, the number of live and dead pups per group on lactation day 0 and pub survival after lactation day 0. All analyses utilized two-tailed tests for a minimum significance level of 5% comparing the control to the treated groups.

Test Conditions: 12 male rats (195-242 g, 6 weeks old) per group were exposed for 28 days prior to mating, and through mating until euthanasia for a total of 44 consecutive days of dosing; 12 females (163-219 g, 8 weeks old) per group were dosed for 14 days prior to mating, during mating, gestation and lactation through euthanasia at lactation day 4 [41-55 consecutive days]. Dose levels were 0, 100, 500, 1000 mg/kg/day in a corn oil vehicle [5 mL/kg]. Viability and development of the pups were followed through lactation day 4. Animals were observed daily for clinical signs of toxicity. Body weights and food consumption were determined weekly. Each male was cohabited with one female and observed daily for evidence of copulation. If no evidence of copulation was confirmed after 10 days of cohabitation, the female was separated from the first male and placed with a second (proven) male for a maximum of 5 days. Females that delivered were necropsied on lactation day 4. Females that failed to deliver were necropsied 25 days after evidence of mating was detected. For females, the number of uterine implantation scars was recorded and the ovaries and brain were weighed. For males, after 43 days of dosing, the viscera were examined, and brain, testes and epididymides weighed. The ovaries, testes, epididymides, liver, kidneys, and peripheral (sciatic) nerve of control and high dose animals, the kidneys of the low and mid dose animals, and all gross lesions from each group, were processed for microscopic examination. Pup viability, weight, and a detailed examination of the pups including sex determination, were performed on lactation days 0 and 4. All intact pups dying prior to lactation day 4 were necropsied and examined with emphasis on developmental morphology.

Results

NOEL: NOEL for maternal toxicity >1000 mg/kg
NOEL for developmental toxicity > 1000 mg/kg/day

Actual dose received by dose level by

sex if known:	As administered. Analysis of dosing mixtures confirmed that mixtures were accurately prepared.
Remarks:	There was no evidence of developmental toxicity in the F1 generation, as measured by the number of live and dead pups, number of litters with live offspring, mean litter size and male to female pup ratio, pup survival and weights, and external observations. No mortality or clinical signs of toxicity were observed. For the F0 males and females at the top dose, gross and histological examination of the ovaries , testes, epididymides, liver, kidneys , and peripheral [sciatic] nerve was performed; kidneys were also examined at the mid and low dose levels. The only gross finding was pitted kidneys in a few mid and top dose males, and the only histological finding was dose-related accumulations of hyaline droplets in the epithelial cells of the convoluted tubules of the kidneys of males; no such effect was observed in female rats. This condition was diagnosed as hydrocarbon nephropathy, which is considered specific to young adult male rats; there is no indication that similar nephropathy will occur in humans exposed to 1-hexene.
Reliability:	(1) Reliable without restrictions
Flag:	Key study for SIDS endpoint
References:	Gingell, R., Daniel, E.M., Machado, M, and Bevan, C. (2000) Reproduction/Developmental Toxicity Screening Test in Rats with Orally-Administered 1-Hexene. Drug and Chem. Toxicology 23(2)327-338.
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added. This study also appears in Section 5.9.A(2), Fertility and Section 5.5.D, Repeated Dose Toxicity.

5.10 Other Relevant Information

A. Aspiration

Test Substance

Identity:	C6-C18 even numbered alpha olefins

Method

Type:	General toxicity – aspiration
Species:	Rat

Strain:	Wistar
Sex:	Male
Route of Administration:	aspiration
Dose:	0.2 mL
Results:	See Remarks
Remarks:	C6-C18 alkenes (even carbon numbers, alpha olefins), source and purity unspecified, were assessed for aspiration hazard in an animal study using Wistar rats. Four or five males were used per test article. Two-tenths mL of the test material was placed in the mouths of rats that had been anesthetized to the point of apnea in a covered wide mouth gallon jar containing about 1 inch of wood shavings moistened with approximately 1 ounce of anhydrous diethyl ether. As the animals began to breathe again, the nostrils were held until the test material had been aspirated or the animal regained consciousness. All alkenes tested except 1- hexene were aspirated into the lungs. 1-Hexene was difficult to dose because of its volatility. Two animals survived because the hydrocarbon "boiled" out of the mouth before it was aspirated. All animals exposed to C_8 to C_{14} died within 24 hours. With C_{16} and C_{18}, there was only one death (C_{18}). Lung weights were increased in alkenes-treated animals compared with controls. The affected animals showed chemical pneumonitis. The report concluded that there is a significant aspiration hazard with C_6 to C_{14} alkenes.
Reference:	Gerarde, H.W. (1963) Toxicological Studies on Hydrocarbons. Archives of Environmental Health 6:329-341.
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

B. Neurotoxicity

(1) Test Substance

Identity:	CAS No. 68526-52-3, Alkenes C6 , internal branched
Remarks:	Composition: C5 n-olefins = 0.5%, C5 iso-olefins = 1.3%, C6 n-olefins = 10.4%, C6 iso-olefins = 55.6%, C5 n-paraffins = 3.3%, C5 iso-paraffins = 9.3%, C6 iso-paraffins = 17.8%, C7 iso-olefins = 1.0%

Method

Method/guideline:	OECD 422
Test type:	Combined repeated dose toxicity study with reproduction/developmental toxicity screening test
GLP:	Yes
Year:	2002

Species:	Rat
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR
Route of Administration:	Oral gavage
Duration of test:	Up to 38 days (general systemic toxicity and neurotoxicity), see Remarks
Concentration levels:	0, 100, 500, or 1000 mg/kg b.w./day
Sex:	Males and females
Exposure period:	Minimum of 28 days and up to 38 days; see remarks
Frequency of treatment:	Once daily
Control group and treatment:	Concurrent vehicle control (corn oil)
Statistical methods:	Data for the toxicity study, including body weights, body weight gain, food consumption, were analyzed by One-Way Analysis of Variance (ANOVA). If significance was detected ($p<0.05$), pairwise group comparisons were performed using the Tukey-Kramer test. Descriptive (categorical) data and quanta data were analyzed by Fisher's Exact Test. When significance was observed, group by group comparisons were performed using Fisher's Exact Test. All analyses were two-tailed with a minimum significance level of 5% ($p<0.05$).
Test Conditions:	This study was conducted to: (1) provide screening information on the repeated-dose systemic toxicity of the test substance, with emphasis on potential neurological effects, and (2) serve as a screening study for potential reproductive and developmental effects in male and female rats. GENERAL TOXICITY AND NEUROTOXICITY PHASE: See Section 5.5.A for complete test conditions for the general toxicity and neurotoxicity phase. For the neurotoxicity evaluation, an abbreviated functional observation battery (FOB), including home cage, removal from home cage and open field evaluation, was performed prior to study initiation and weekly thereafter. A full FOB, including home cage, removal from home cage, open field evaluation, manipulative tests and motor activity measurements, was performed following 28 days of treatment., An open field chamber (San Diego Instruments, San Diego, CA) was used for the motor activity assessment. For each animal, the test consisted of a one-hour observation period in the chamber, under red lighting, with white noise from a SPER Scientific Sound Meter, Model 840029 (73-74 dB).

Results

NOAEL (NOEL):	NOEL (neurotoxicity) = 1000 mg/kg/day (reviewer assigned)
Actual dose received by dose level by	

sex if known:	As administered. Analysis of dosing mixtures confirmed that mixtures were accurately prepared.
Remarks:	No statistically significant or toxicologically meaningful differences were noted in the functional observation battery evaluations. See Section 5.5A for general systemic toxicity results, and Sections 5.9.A (1) and 5.9.B(1) for reproductive toxicity results.
Reliability:	(1) Reliable without restrictions.
Flag:	Key study for SIDS endpoint.
References:	Thorsrud, B.A. (2003) A combined repeated dose toxicity study and reproduction/developmental screening study in Sprague Dawley rats with (C6) alkenes, Study No. 3604.2, Springborn Laboratories, Inc., Ohio Research Center, Spencerville, Ohio; conducted for American Chemistry Council (Higher Olefins Panel) (unpublished report).

(2) Test Substance

Identity (purity):	CAS No. 592-41-6, 1-Hexene (90 – 100%, NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 413
Test type:	90-day subchronic inhalation toxicity study
GLP:	Yes
Year:	1984
Species:	Rat
Strain:	F344
Route of Admin.:	Inhalation – vapor
Duration of test:	90 days
Doses:	0, 300, 1000, 3000 ppm (0, 1033, 3442, 10,326 mg/m^3)
Sex:	Males and females
Exposure period:	6 hr/day
Freq. of treatment:	5 days/week, 13 weeks
Control group:	Air exposed
Post exposure observation period:	Not applicable
Statistical methods:	The Rotorod data for neuromuscular coordination were adjusted by summing the time for the best 3 of 4 trials for each rat.
Test Conditions:	See Section 5.5.B

Results

NOAEL (NOEL):	NOAEL = 3000 ppm (neurotoxicity)

Actual dose received by dose level by sex (if known):	0, 300, 1000, 3000 ppm
Remarks:	Exposure to 1-hexene did not affect neuromuscular coordination in females as determined using the Rotorod.
Reliability:	(1) Reliable without restrictions
References:	Gingell, R., Bennick, J.E., and Malley, L.A. (1999) Subchronic inhalation study of 1-hexene in Fischer 344 rats. Drug Chem Toxicol. 22(3):507-28.
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

(3) Test Substance

Identity (purity):	CAS No. 592-41-6, 1-Hexene (90 – 100%, NEODENE 6 alpha olefin)

Method

Method/guideline:	OECD 407
Test type:	28-day oral repeated dose study
GLP:	Yes
Year:	1994
Species:	Rat
Strain:	Wistar
Route of Admin.:	Oral gavage
Duration of test:	28 days
Doses:	0, 10, 101, 1010, 3365 mg/kg/day
Sex:	Males and females
Exposure period:	28 days
Freq. of treatment:	daily for 28 days
Control group:	Dosed with water
Post exposure observation period:	none
Statistical methods:	No data
Test Conditions:	See Section 5.5.C

Results

NOAEL (NOEL):	NOEL = 3365 mg/kg/day (neurotoxicity)
Actual dose received by dose level by sex (if known):	No data

Remarks:	Neuromuscular coordination [by rotorod] were unaffected.
Reliability:	(1) Reliable without restrictions
References:	Dotti, A., Duback-Powell, J.R., Biderman, K., and Weber, K. (1994) 4-week oral toxicity (gavage) study with 1-hexene in the rat. RCC Project 332695. Cited in HEDSET.
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

5.11 Experience with Human Exposure

Test Substance:	CAS No. 592-41-6, 1-Hexene
Remark:	In a review, Cavender (1998) noted that 1-hexene, when inhaled, may produce narcosis in humans at a concentration of 0.1 percent with accompanying CNS effects, mucous membrane irritation, vertigo, vomiting and cyanosis.
Reference:	Cavender, F (1998). Aliphatic Hydrocarbons. In: Patty's Industrial Hygiene and Toxicology. CD-ROM. Vol. 2B, Chapter 19. Edited by Clayton GD, Clayton FE, Cralley LJ, Cralley LV, Harris RL and Bus JS. John Wiley & Sons, Inc., 1249.
Other:	This study was included in the dossier for 1-hexene at SIAM 11. Additional information has been added.

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